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Fluorinated Olefinic Peptide Nucleic Acid: Synthesis and Pairing Properties with Complementary DNA

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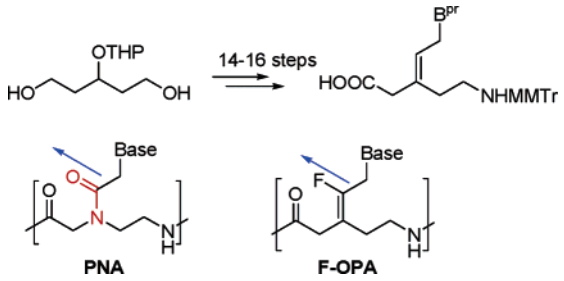

The fluorinated olefinic peptide nucleic acid (F-OPA) system was designed as a peptide nucleic acid (PNA) analogue in which the base carrying amide moiety was replaced by an isostructural and isoelectrostatic fluorinated C−C double bond, locking the nucleobases in one of the two possible rotameric forms. By comparison of the base-pairing properties of this analogue with its nonfluorinated analogue OPA and PNA, we aimed at a closer understanding of the role of this amide function in complementary DNA recognition. Here we present the synthesis of the F-OPA monomer building blocks containing the nucleobases A, T, and G according to the MMTr/Acyl protecting group scheme. Key steps are a selective desymmetrization of the double bond in the monomer precursor via lactonization as well as a highly regioselective Mitsunobu reaction for the introduction of the bases. PNA decamers containing single F-OPA mutations and fully modified F-OPA decamers and pentadecamers containing the bases A and T were synthesized by solid-phase peptide chemistry, and their hybridization properties with complementary parallel and antiparallel DNA were assessed by UV melting curves and CD spectroscopic methods. The stability of the duplexes formed by the decamers containing single (*Z*)-F-OPA modifications with parallel and antiparallel DNA was found to be strongly dependent on their position in the sequence with T_m values ranging from +2.4 to −8.1 °C/modification as compared to PNA. Fully modified F-OPA decamers and pentadecamers were found to form parallel duplexes with complementary DNA with reduced stability compared to PNA or OPA. An asymmetric F-OPA pentadecamer was found to form a stable self-complex (T_m ∼ 65 °C) of unknown structure. The generally reduced affinity to DNA may therefore be due to an increased propensity for self-aggregation.

Introduction

The peptide nucleic acids (PNAs) are neutral, achiral DNA mimics that bind to complementary oligonucleotides with high affinity and sequence specificity.[1] PNA was originally designed to bind and recognize double-stranded DNA in the major groove via triple helix formation.[2] The peptide backbone has proven to be a very good structural mimic of the ribose−phosphate backbone of nucleic acids and was shown to bind with higher affinity to complementary nucleic acids than their natural counterparts, obeying the Watson−Crick base-pairing rules.[3,4] Therefore, PNA has attracted wide attention in medicinal chemistry for the development of gene therapeutic (antisense and antigene) drugs and in DNA diagnostics.[5−8] Unfortunately, therapeutic applications of PNA have not

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been realized to date due to incompatibilities beyond target binding. These comprise among others poor cellular uptake,[9−11] low water solubility,[2,4,12,13] and a tendency for self-aggregation.[4,14,15] As a consequence, a variety of PNA analogues have been introduced in the recent years.[16,17] Among them is the aminoethylprolyl (aep) PNA that seems to be a potent PNA surrogate, since it showed considerable hybridization stabilization, at least in the triplex-binding mode.[18−20] Further modifications of the PNA skeleton include pyrrolidinyl PNA,[21,22] which displays a remarkable preferential affinity for complementary DNA over RNA; cyclopentane PNA;[23] and finally, the G-clamp containing PNAs,[24,25] both of which lead to strong improvements in RNA and DNA affinity. PNAs can also readily be derivatized by attachment of peptides, in order to improve the cellular uptake properties;[11,26,27] by attachment of fluorophores,[28,29] to yield tools in diagnostics; or by introduction of linkers, for applications in microarray technology.[30]

An interesting structural feature of PNA is the central amide linkage connecting the nucleobases to the backbone. The carbonyl oxygens of these units uniformly point toward the carboxy termini in PNA/DNA duplexes,[31,32] PNA/DNA triplexes,[33] PNA/RNA duplexes,[34] PNA/PNA duplexes,[35] and duplexes of modified PNA with PNA or

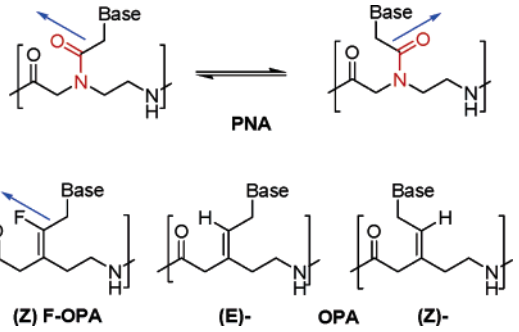

FIGURE 1. Chemical structure of the two rotameric forms of PNA (top) and of the (*Z*)-F-OPA as well as the (*E*)- and (*Z*)-OPA (bottom). The central amide is highlighted in red and the dipole moment of the carbonyl group is schematically represented with a blue arrow.

natural DNA.[36−38] However, the free monomers along with unbound dimers and octamers were shown by NMR spectroscopy and molecular dynamics to coexist as mixtures of the cis and trans rotamers and to interconvert slowly on the NMR time scale.[39] Early molecular mechanics calculations[40,41] suggested that inter-residue hydrogen bonding between the backbone and the central amide linkage might account for this structural feature and for the stability of the helical structures formed by PNA, a notion that was not supported by the structural analyses.

To remove the structural ambiguity of the rotameric forms in PNA and in order to probe the impact of the central amide function on complementary DNA recognition, we recently designed the olefinic peptide nucleic acid (OPA) system, in which this structural feature was replaced by an isostructural, configurationally stable C−C double bond in either the *Z* or *E* configuration (Figure 1).[42]

Compared to PNA, fully modified (*E*)-OPA oligoamides (isostructural to PNA in complexed form) showed a drop in stability and an inverted preference for parallel strand orientation in duplexes with complementary DNA.[42] In contrast to PNA, fully modified OPA sequences (*E* and *Z*) were not able to form triple helical structures. The OPA system thus revealed that the amide functionality in the base-linker unit in PNA significantly determines the affinity and preferred strand orientation in PNA/DNA

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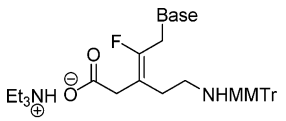

1 Base = thy
2 Base = ade^{6}Bz
3 Base = gua^{2}iBu

FIGURE 2. Chemical structures of the thyminyl-, adeninyl-, and guaninyl-F-OPA monomeric units used in oligopeptide synthesis according to the MMTr/Acyl protecting group scheme.

duplexes. Furthermore, this linkage seems to be responsible for the propensity to form $(PNA)_2$·DNA triplexes.

(*Z*)-F-OPA, in turn, was designed to restore the dipolar nature of the linker−carbonyl group in PNA while the structural constraints of the OPA system were maintained (Figure 1). With this we intended to assess the importance of the electrostatic factor of this unit on the helix-forming properties.[43] The synthesis of the (*Z*)-thyminyl-F-OPA monomeric unit **1** and its incorporation into PNA oligoamides has recently been reported in preliminary form.[43] Here, we describe the synthesis of the adenine- and guanine-containing (*Z*)-F-OPA monomeric units (Figure 2). The incorporation of single (*Z*)-adeninyl-F-OPA units into PNA oligoamides and the assessment of their pairing properties are reported. Finally, fully modified (*Z*)-F-OPA oligoamides containing the bases adenine and thymine were synthesized and their hybridization properties investigated by UV melting curves and CD spectroscopy. The results were compared and discussed with those of the corresponding OPA- and PNA-oligoamides.

Results and Discussion

Synthesis of the (*Z*)-Adeninyl-F-OPA Monomeric Unit 2. The synthesis of the adeninyl-F-OPA monomer **2** started from the intermediate diol **11**, which had already been used before in the preparation of the F-OPA thyminyl monomer.[43] The slightly changed and optimized synthesis of **11** starts from the known THP-protected triol **4**,[44] which was obtained in two steps from the commercially available diethyl 3-hydroxyglutarate (Scheme 1).

Diol **4** was bis-benzylated and subsequently THP-deprotected to yield **6**. Intermediate **6** was then oxidized to ketone **7** under mild conditions using *o*-iodoxybenzoic acid (IBX)[45] and converted to the α-fluoro ester **8** via a Horner−Wadsworth−Emmons reaction.[46] No products arising from *β*-elimination of the OBn moieties as a side reaction in this step could be isolated. A BCl_3-mediated deprotection−lactonization step of intermediate **8** then led to lactone **9** in good yields and with this to the desired desymmetrization of the double bond. Lactone **9** was then TBDMS-protected and reductively opened under Luche's conditions, leading to diol **11**.

The introduction of the adenine nucleobase was planned via a Mitsunobu reaction on diol **11**.[43] We reasoned that the substitution of the primary hydroxyl function occurs preferentially in the allylic as compared to the homoallylic position, due to enhanced acidity and/or the assistance of the neighboring *π*-system. Indeed, only allylic displacement was observed in all cases investigated. Different N-protected adenine derivatives were tested in order to optimize N^9/N^7 regioselectivity in this Mitsunobu reaction, however, with limited success. Only 6-chloropurine performed acceptably in this context (Scheme 2).

Compound **12** was thus obtained and its constitution (N^9-attachment of the base) was unambiguously confirmed by NMR ($^{13}C/^{1}H$ HMBC and 2D T-ROESY) (see Supporting Information). Intermediate **12** was then converted into the adenine derivative **13** by treatment with concentrated NH_3 in methanol at 70 °C in a sealed high-pressure vessel. Intermediate **13** was subsequently subjected to an Appel reaction,[47] which led to azide **14** in good yields. The exocyclic amino function of the base in **14** was benzoylated using benzoyl chloride followed by mild ammonia treatment to remove one of the two initially introduced benzoyl groups. The silyl ether function of intermediate **15** was then cleaved using the pyridine−HF complex in good yields to furnish **16** that was oxidized in a two-step procedure to the carboxylic acid **17**. First, **16** was oxidized to the corresponding aldehyde using the Dess−Martin periodinane[48] and then further oxidized with sodium chlorite in *t*-BuOH, to acid **17**.[49,50] Successful reduction to the amino acid was accomplished by a Staudinger reaction of azide **17** with triphenylphosphine in pyridine, followed by hydrolysis of the intermediate phosphinimine with NH_3.[50] Subsequent MMT protection of the amino acid afforded the (*Z*)-adeninyl-F-OPA monomeric building block **2**.

Synthesis of the (*Z*)-Guaninyl-F-OPA Monomer 3. Following the same strategy as described above, diol **11** was coupled with O^6-diphenylcarbamoyl-N^2-isobutyrylguanine[51] and afforded **18** in good yield (Scheme 3). Also in this case the N^9-attachment of the base was unambiguously assigned by $^{1}H/^{13}C$ HMBC NMR spectroscopy. The azide function was again introduced by applying the Appel conditions as used before. The diphenylcarbamoyl protecting group neatly departed under these conditions and azide **19** could directly be isolated in good yields. Removal of the silyl protecting group and oxidation of the resulting hydroxyl group as previously described, afforded acid **20**. Reduction of the azide to the amine and subsequent MMTr-protection of the amino functionality finally led to the monomer **3**.

Synthesis of Oligoamides. The synthesis of oligoamides was carried out by application of the MMTr/acyl strategy developed for PNA−DNA chimeras.[52] This method has been applied successfully in the synthesis of OPA oligoamides in the past.[42] The automated syntheses started with the glycine-containing CPG-solid support **22**

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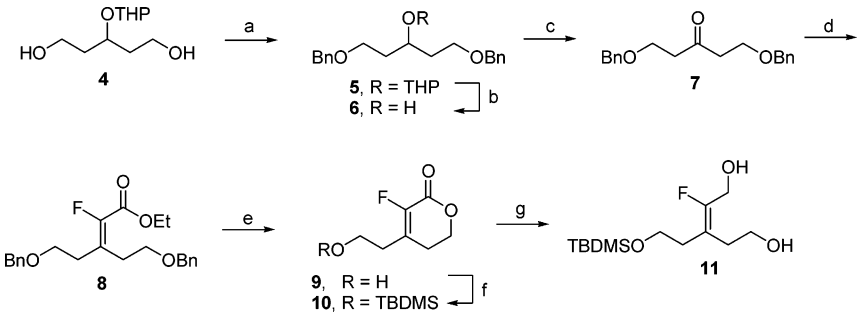
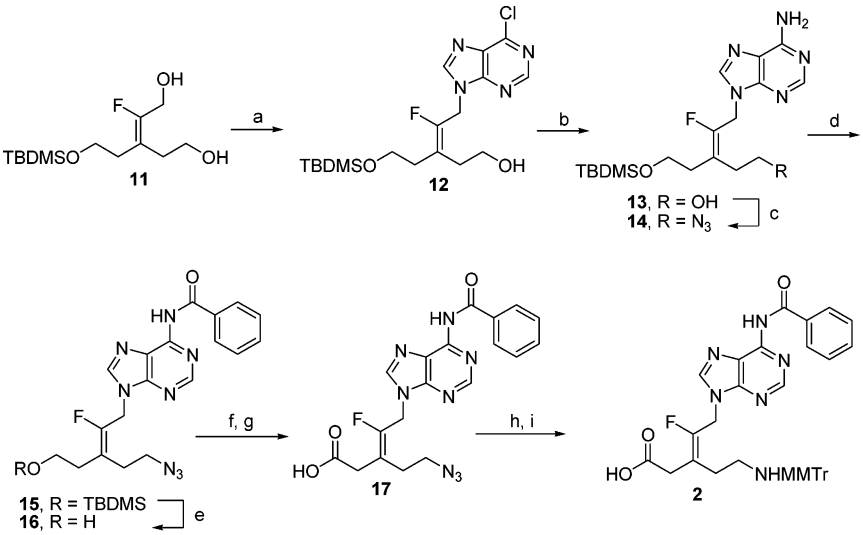
SCHEME 1. Synthesis of Diol 11[a]



[a] Reagents and conditions: (a) NaH, THF, 0 °C → rt, 1 h, then, BnBr, THF, rt, 12 h, 67%; (b) PpTS, EtOH, 55 °C, 3 h, 94%; (c) IBX, THF:DMSO = 1:1, rt, 4 h, 92%; (d) NaH, $(EtO)_2P(O)CHFCO_2Et$, THF, −78 °C, 1.5 h, then **7**, −78 °C → rt, 5 h, 75%; (e) BCl_3, CH_2Cl_2, −15 °C → rt, 3 h, 95%; (f) TBDMSCl, imidazole, DMF, rt, 12 h, 99%; (g) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, methanol, 0 °C → rt, 4.5 h, 94%.

SCHEME 2. Synthesis of (Z)-Adeninyl-F-OPA 2[a]



[a] Reagents and conditions: (a) 6-chloropurine, DIAD, PPh_3, DMF, rt, 12 h, 34%; (b) NH_3 (sat.)/MeOH, 70 °C, 12 h, 84%; (c) LiN_3, PPh_3, CBr_4, DMF, 0 °C → rt, 12 h, 79%; (d) BzCl, DMAP (cat.), pyridine, 0 °C → rt, 12 h, then NH_3 (concd), 1 h, rt, 66%; (e) HF·pyridine, THF, rt, 12 h, 95%; (f) Dess−Martin periodinane, CH_2Cl_2, rt, 2 h; (g) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, rt, 12 h, 59% (over 2 steps); (h) PPh_3, pyridine, concd NH_3, rt, 3 h; (i) MMTrCl, pyridine, rt, 12 h, 53% (over 2 steps).

(Scheme 3)[42] and were performed on a 1-μmol scale using a Pharmacia Gene Assembler DNA synthesizer.

During optimization of the reaction conditions, we found that the detritylation kinetics for F-OPA units was much slower than that for PNA. This finding forced us to use stronger acidic conditions (TFA). The original coupling and capping conditions that were applied for the synthesis of OPA oligoamides were left untouched, since high coupling yields were obtained. Moreover, capping of the free amine moieties was carried out using isobutyric anhydride in order to avoid partial capping of the MMTr-protected amino termini as observed with acetic anhydride.[53] Lysine residues were incorporated either at the *N*- or *C*-terminus or both in order to increase solubility and suppress self-aggregation.[4] The coupling yields for the F-OPA units were in the range 94−100%, as determined by the trityl assay. The optimized synthesis cycle for all three systems (PNA, OPA, and F-OPA) is indicated in the Supporting Information (Scheme S1).

Detachment and base deprotection were first tried by standard treatment with concentrated NH_3 (55 °C, 12 h) and were found to result in massive degradation of the PNA and F-OPA oligoamides. For example, application of these conditions to the thyminyl F-OPA trimer **23** (vide infra) led to cleavage of the *N*-terminal unit via intramolecular aminolysis, resulting in the production of the fragments **24** and **25**, which could be isolated and characterized by ESI^+-MS spectrometry (Figure 3, data not shown). Intramolecular aminolysis under basic conditions had already been reported for PNA with unmodified N-termini.[4,54] We found that degradation under these conditions also occurred with F-OPAs and PNAs that were modified with a lysine group at the *N*-terminus. Milder deprotection conditions (7 N NH_3 in MeOH, rt, 2 h) or shorter exposure times (2 h, rt) to 33% NH_4OH reduced the extent of degradation but compromised with the extent of base deprotection, especially in the case of guanine.[55] Alternatively, we found that treatment with 33% $MeNH_2$ at room temperature for 6 h led to detachment of the oligoamide from the solid support and

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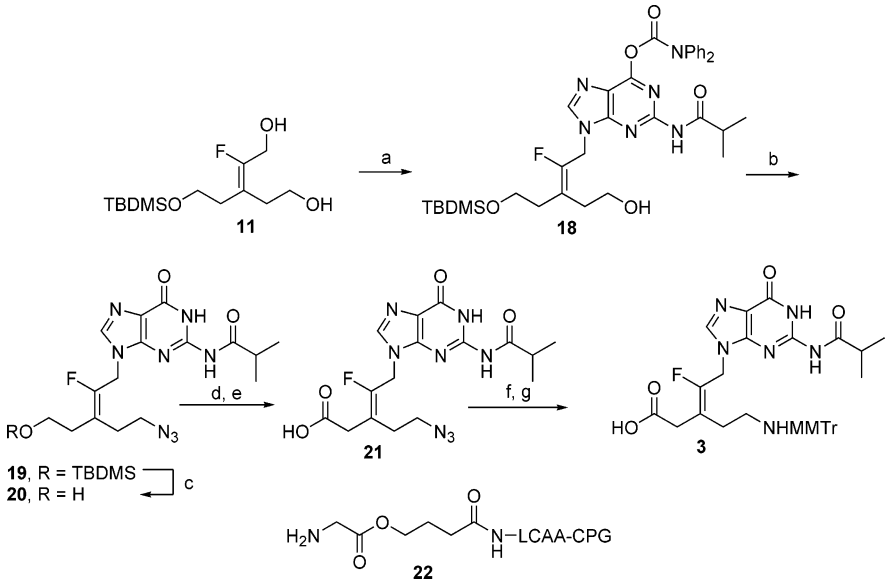
SCHEME 3. Synthesis of the (Z)-Guaninyl-F-OPA 20[a]



[a] Reagents and conditions: (a) O^6-diphenylcarbamoyl-N^2-isobutyrylguanine, DIAD, PPh_3, DMF, rt, 12 h, 59%; (b) LiN_3, PPh_3, CBr_4, DMF, 0 °C → rt, 12 h, 64%; (c) HF·pyridine, THF, rt, 12 h, 100%; (d) Dess−Martin periodinane, CH_2Cl_2, rt, 2 h; (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, rt, 12 h, 41% (over 2 steps); (f) PPh_3, pyridine, concd NH_3, rt, 3 h; (g) MMTrCl, pyridine, rt, 12 h, 65% (over 2 steps).

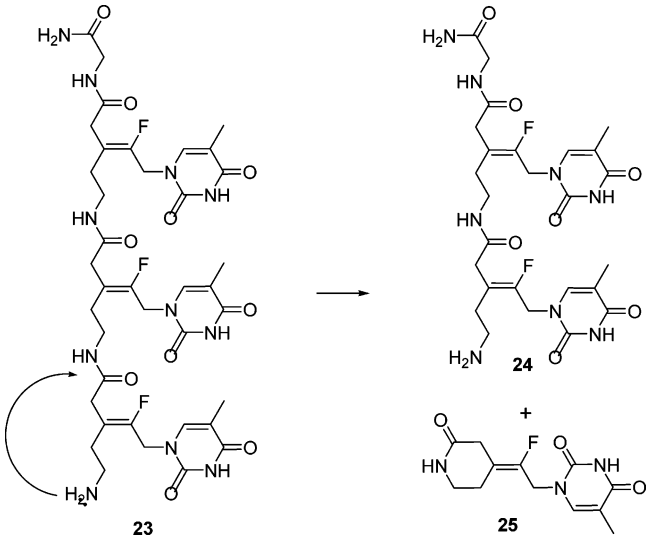


FIGURE 3. Ammonia-mediated cleavage of trimer **23**.

deprotection of the adenine residues without extensive aminolysis, leaving to oligoamides with a methylamide C-terminus. Unfortunately, under all the milder conditions tested, the deprotection of the guanine residues remained incomplete. An adequate protecting group allowing also for the incorporation of (*Z*)-guaninyl-F-OPA units into oligoamides is thus still elusive. The oligoamides were then purified by reverse phase HPLC and their identities were routinely confirmed by ESI^+ mass spectrometry.

NMR Structural Analysis of the Trimer $t^Ft^Ft^F$-Gly-NH_2 (t^F = thyminyl-F-OPA) (23). One concern in the synthesis of F-OPA according to Scheme 3 was the constitutional and configurational stability of the double bond under the conditions used for coupling and MMTr-deprotection, as well as oligoamide detachment and deprotection. To test the propensity of the C−C double bond to migrate into α,β-insaturation, trimer **23** and dimer **24** were synthesized, isolated, and analyzed by NMR spectroscopy (Figure 4).

The fluorine atoms and the allylic protons next to the bases (inset, Figure 4, left), which present characteristic chemical shifts and coupling patterns, acted as spectroscopic probes. The ^1H and ^{19}F NMR spectra in D_2O of the trimer **23** and the dimer **24** (see Supporting Information) showed only the expected ^1H and ^{19}F NMR signals and thus ruled out double bond migration under the conditions used for oligoamide synthesis and deprotection.

Duplex Formation of PNAs Containing Single F-OPA Units with Complementary DNA. Incorporation of (*Z*)-thyminyl-F-OPA **1** and (*Z*)-adeninyl-F-OPA **2** units into a PNA sequence displaying an asymmetric base distribution was effected in order to gain insight on the effect of F-OPA units on the hybridization properties. All synthesized oligoamides and their binding properties with antiparallel and parallel DNA are summarized in Table 1.

Pairing to Antiparallel DNA. Sequences **28**−**34**, comprising single thyminyl-OPA and (*Z*)-thyminyl-F-OPA units, showed a strong dependence of stability from the position of the modifications within the sequence when paired to complementary antiparallel DNA.[43] Indeed, the introduction of the (*Z*)-thyminyl-F-OPA mutation in the center of the sequence, flanked by two adenine bases (oligoamide **30**), led to a stabilization of the antiparallel duplex (ΔT_m = +2.4 °C) compared to unmodified PNA **26**. Positioning of the modification between an adenine and a thymine nucleobase, as in sequence **32**, led to a decreased T_m (ΔT_m = −6.2 °C) and insertion of the mutation between two thymine bases (sequence **34**) caused a marked drop in stability (ΔT_m = −8.1 °C). The reference (*E*)-thyminyl-OPA series undergoes similar

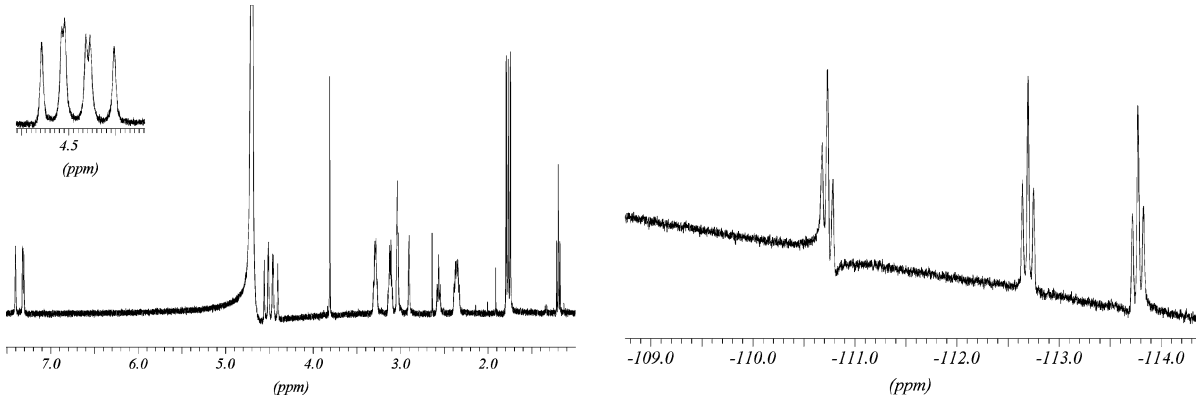


FIGURE 4. ^{1}H NMR (D_2O, 500 MHz) of trimer **23** (left) and ^{19}F NMR (376.5 MHz, D_2O) of trimer **23** (right).

TABLE 1. T_m **Data (°C, 260 nm) of Decamers Containing Single Modifications**[a]

sequence[b]	d(TATATTAAAA) (antiparallel)	d(AAAATTATAT) (parallel)
26, Lys-ttttaatata-Gly-NH_2	33.2[c]	11.6
27, Lys-ttttaatata-Lys-Gly-NH_2	36.7[c]	16.3
28, Lys-ttttaat^{E}ata-Gly-NH_2	36.7[c]	13.1
29, Lys-ttttaat^{Z}ata-Gly-NH_2	28.0[c]	6.1
30, Lys-ttttaatFata-Gly-NH_2	35.6[c]	13.0
31, Lys-ttt^{E}aatata-Gly-NH_2	30.0[c]	11.0
32, Lys-ttt^{F}aatata-Gly-NH_2	27.0[c]	8.0
33, Lys-tt^{E}taatata-Gly-NH_2	28.1[c]	9.1
34, Lys-tt^{F}taatata-Gly-NH_2	25.1[c]	9.0
35, Lys-ttttaata^{F}ta-Lys-Gly-NH_2	34.0	9.0
36, Lys-tttta^{F}atata-Lys-Gly-NH_2	31.7	8.0

[a] Total oligonucleotide concentration: 4 μM in 100 mM NaCl, 10 mM Na_2HPO_4, pH 7.0; Heating rate: 0.5 °C/min. [b] Capital letters, 2′-deoxynucleotides; lowercase letters, PNA units; t^Z, (*Z*)-OPA unit, t^E, (*E*)-OPA unit; t^F, t-F-OPA; a^F, a-F-OPA. [c] Data from ref 43.

though less marked sequence effects (see sequences **28**, **31**, and **33**).

The T_m values of oligoamides **35** and **36** bearing one (*Z*)-adeninyl-F-OPA unit reflected a similar behavior as observed in the thymine series, however with inverted nearest neighbor preference. Here, positioning of the mutation between two thymine nucleobases, as in sequence **35**, led to a slight destabilization ($\Delta T_m = -2.7$ °C) compared to native PNA **27**. The T_m value, however, significantly dropped ($\Delta T_m = -5.0$ °C) upon positioning the modification between one adenine and one thymine base, as in sequence **36**. Due to the choice of the sequence, it was not possible to assess the effect of two adenine PNA residues flanking a (*Z*)-adeninyl-F-OPA unit.

Pairing to Parallel DNA. A similar trend was observed upon pairing of these single (*Z*)-thyminyl-F-OPA containing PNA oligoamides to complementary parallel DNA. Indeed, positioning of a single (*Z*)-thyminyl-F-OPA mutation between two adenine nucleobases, as in **30**, led to an increase in duplex stability ($\Delta T_m = +1.4$ °C) as compared to the unmodified PNA strand **26**. Introduction of this mutation between a thymine and an adenine (sequence **32**) or between two thymine bases (sequence **34**) led to a decrease in duplex stability ($\Delta T_m = -2.6$ °C and -1.6 °C, respectively). However, unlike in the antiparallel series, the order of stability was inverted and the oligoamide **34**, in which the modification was comprised between two thymine bases, led to a smaller destabilization as compared to oligoamide **32**. The variation of the stability of the oligoamides containing (*E*)-thyminyl-OPA single mutations as a function of their position in the sequence strictly followed the pattern met for the antiparallel hybrids. Indeed, PNA oligomer **28**, containing the (*E*)-thyminyl-OPA unit in a central position of the sequence, showed a stabilization of the duplex ($\Delta T_m = +1.5$ °C) compared to unmodified PNA **26**. When the (*E*)-thyminyl-OPA was flanked by an adenine and a thymine base as in sequence **31**, an intermediate T_m value was obtained ($\Delta T_m = -0.6$ °C), and when the mutation was placed between two thymine bases, as in sequence **33**, a marked destabilization was observed ($\Delta T_m = -2.5$ °C). Introduction of a single (*Z*)-thyminyl-OPA unit as in sequence **29**, causing a geometric mismatch in the base-orientation, resulted in ΔT_m of -5.5 °C.

The destabilization of the parallel duplex caused by one (*Z*)-adeninyl-F-OPA unit embedded in the middle of the sequence (PNA **35**) was more severe than the loss of duplex stability caused by inserting a (*Z*)-thyminyl-F-OPA unit between two thymine residues ($\Delta T_m = -7.3$ °C as compared to **27**). Positioning of one (*Z*)-adeninyl-F-OPA unit between one thymine and one adenine nucleobase led to a duplex of even poorer stability when paired to complementary parallel DNA ($\Delta T_m = -8.3$ °C).

This study on PNAs containing single (*Z*)-F-OPA units thus showed that the nucleobases in the F-OPA system are constrained in a conformation that in principle allows for Watson−Crick base pairing. This conclusion is based on the fact that there exist OPA mutations in PNA that give rise to almost equal or even enhanced stabilities compared to standard PNA−DNA duplexes. The observed sequence effects are likely associated with differential stacking. As far as single modified oligoamides are concerned, the F-OPA system appears therefore to be a reasonable structural PNA surrogate.

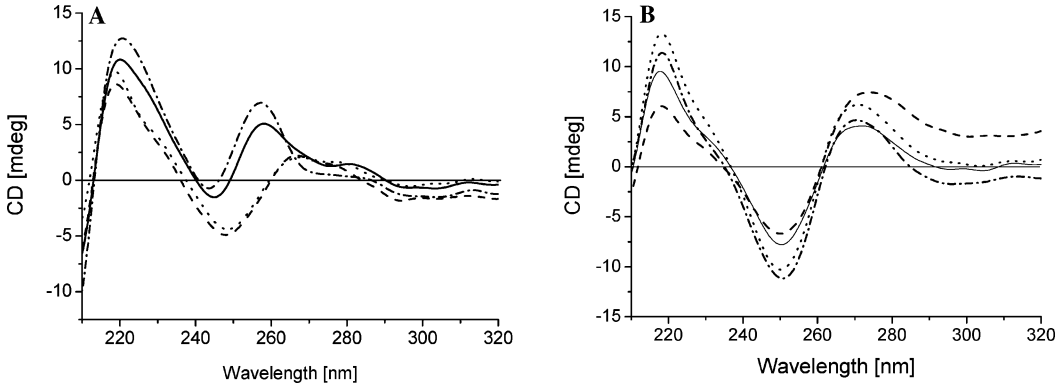


FIGURE 5. CD spectra of PNA·DNA duplexes. (A) (-·-) Lys-ttttaatata-Lys-Gly, **27**; (···) Lys-ttt**t**^{F}aatata-Gly-NH_2, **32**; (--) Lys-ttttaat**a**^{F}ta-Lys-Gly-NH_2, **35**; (—) Lys-ttt**t**^{E}aatata-Gly-NH_2, **31**; paired with antiparallel DNA. (B) (-·-) Lys-ttttaatata-Lys-Gly, **27**; (···) Lys-ttt**t**^{F}aatata-Gly-NH_2, **32**; (--) Lys-ttt**t**^{E}aatata-Gly-NH_2, **31**; (—) Lys-ttttaat**a**^{F}ta-Lys-Gly-NH_2, **35**; paired with parallel DNA. Single strand concentration: 4 μM, in 100 mM NaCl, 10 mM Na_2HPO_4, at pH 7.0. All spectra were recorded at 20.0 °C.

TABLE 2. T_m Data (°C, 260 nm) of Homothymine Oligoamides[a]

oligoamides	$d(A)^{10}$	$d(A)^{15}$
37, $d(T)^{10}$	20.7	–
38, Lys-$(t^F)^{10}$-Lys-Gly-NH_2	~0[b]	–
	~3[c]	
39, $(t^Z)^{10}$-Gly-NH_2	23.0[d]	–
40, $(t^E)^{10}$-Gly-NH_2	23.5[d]	–
41, $(t)^{10}$-Gly-NH_2	66.9[d]	–
42, Lys-$(t)^{10}$-Lys-Gly-NH_2	70.6[d,e]	–
43, Lys-$(t^F)^{15}$-Lys-Gly-NH_2	–	~3[b]
44, Ac-$(t)^{15}$-d(NHT)	–	85.0[e]
45, $d(T)^{15}$	–	28.0[e]

[a] Total oligonucleotide concentration 4 μM in 100 mM NaCl, 10 mM Na_2HPO_4, pH 7.0. Heating rate 0.5 °C/min. [b] Single strand concentration 4 μM. [c] Single strand concentration 38.1 μM. [d] Data from ref 42. [e] Data from ref 56, d(NHT) = 5′-aminothymine.

CD Spectra of Single F-OPA-Containing Oligoamide Duplexes. CD spectra were measured under the same conditions as for T_m analysis. The overall shape of the CD spectra of single adenine- and thymine-containing PNA decamers paired to antiparallel DNA is similar to the CD spectral features of native PNA (Figure 5A). However, some changes were noticeable, the most important being the vanishing of the positive Cotton effect observed for the unmodified PNA·DNA complexes at 257 nm and the appearance of an equally strong negative Cotton effect at 248 nm in the case of the monomodified PNAs. On the other hand, the changes in the CD spectral features for the parallel duplexes were minimal (Figure 5B).

Hybridization Properties of Fully Modified Homothymine F-OPA Oligoamides. (*Z*)-F-OPA homothymine strands **38** and **43** were prepared and paired to their DNA complements. The corresponding T_m data are summarized in Table 2. Figure 6 contains representative melting curves of selected hybrids.

As expected, the PNA sequences **41** and **42** formed very stable $(PNA)_2$·DNA triplexes. The hybrids between the (*Z*)- and (*E*)-OPA oligoamides **39** and **40** and $d(A)_{10}$ have very similar T_m values as compared to the pure DNA reference duplex (ΔT_m = +2.3 and +2.8 °C, respectively) but significantly reduced affinity compared to PNA. In contrast to PNA, the OPA homothymine oligoamides were shown previously to form only duplex structures.[42] The F-OPA homothymine decamer **38** showed even

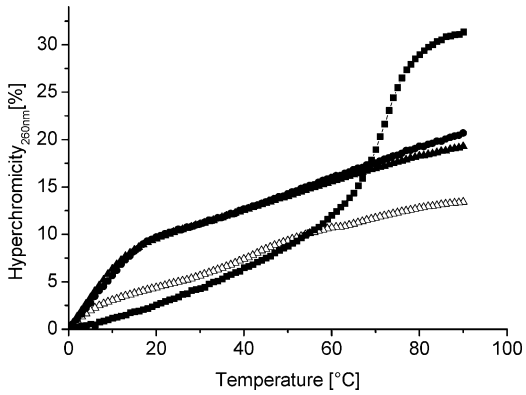


FIGURE 6. Temperature dependence of absorption intensity at 260 nm for equimolar mixtures of (△) Lys-$(t^F)_{10}$-Lys-Gly (**38**):$d(A)_{10}$, single strand concentration 4 μM; (▲) Lys-$(t^F)_{10}$-Lys-Gly (**38**):$d(A)_{10}$, single strand concentration 38.1 μM; (●) Lys-$(t^F)_{15}$-Lys-Gly-NH_2 (**43**):$d(A)_{15}$, single strand concentration 4 μM; (■) Lys-$(t)_{10}$-Lys-Gly-NH_2 (**42**):$d(A)_{10}$. Buffer conditions as indicated in Table 2.

further reduced affinity to $d(A)_{10}$. Under standard conditions (duplex concentrated = 4 μM), no T_m value could be determined. Only upon increasing the concentration by nearly a factor of 10 could a melting transition be observed (T_m ~ 3 °C). The T_m was too low to perform a Job plot analysis for determining the stoichiometry of the complex. In analogy to the OPA system, we tentatively assume that also F-OPA has lost the potential to form triplexes.

Since the T_m value of decamer **38** was quite low, it was surmised that a longer sequence would lead to a more stable complex, thus providing further information on the binding properties of F-OPA homothymine strands. Therefore, pentadecamer **43** was synthesized. Oligoamide **43** formed a duplex upon pairing with complementary DNA of modest stability (T_m ~ 3 °C) compared to the impressive T_m of 85 °C determined for the unmodified PNA sequence Ac-t_{15}-d(NHT) (**44**).[56]

The CD spectra of the hybrids formed between **38** and **43** with the corresponding DNA sequences were virtually identical to those of the DNA single strands (see Supporting Information), hinting at the formation of duplexes of modest stability. Furthermore, the effect of an increase

TABLE 3. T_m **Data (°C, 260 nm) of Oligoamides**[a]

oligoamide	**46**[c]	**47**[c]	**48**[c]	**49**[c]
26, Lys-ttttaatata-Gly-NH_2	33.2	11.6[b]	–	–
27, Lys-ttttaatata-Lys-Gly-NH_2	36.7	16.3[b]	–	–
50, dC(tttttaatata)-Gly-NH_2[d]	29.4	13.1	–	–
51, dC($t^Et^Et^Et^Et^Ea^Ea^Et^Ea^Et^Ea^E$)-Gly-$NH_2$	<0[d]	16.5[d]	–	–
52, Lys-$t^Zt^Zt^Zt^Za^Za^Zt^Za^Zt^Za^Z$-Gly-$NH_2$	nd[e]	21.5[e]	–	–
53, Lys-$t^Ft^Ft^Ft^Fa^Fa^Ft^Fa^Ft^Fa^F$-Lys-Gly-$NH_2$	<0 (44.0)[b]	~6 (38.0)[b]	–	–
54, Lys-taattttaatataat-Lys-Gly-$NHCH_3$[f]	–	–	46.0	38.0
55, Lys-$t^Fa^Fa^Ft^Ft^Ft^Ft^Fa^Fa^Ft^Fa^Ft^Fa^Fa^Ft^F$-Lys-Gly-$NHCH_3$[f]	–	–	nd (65.0)[b]	nd (67.3)[b]

[a] Total oligonucleotide concentration 4 μM in 100 mM NaCl, 10 mM Na_2HPO_4, pH 7.0. Heating rate 0.5 °C/min. [b] The values in parentheses indicate the T_m of the self-complexes. [c] **46** = d(TATATTAAAA), **47** = d(AAAATTATAT), **48** = d(ATTAAAATTATATTA), **49** = d(ATTATATTAAAATTA). [d] Data from ref 42. [e] Data from ref 57. nd = not detected. [f] Methylamine-mediated deprotection and cleavage from the solid support.

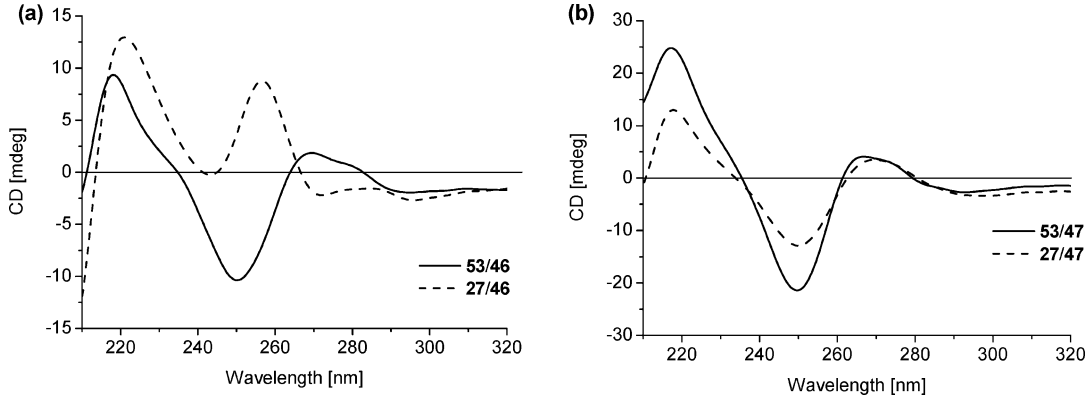


FIGURE 7. CD spectra of oligoamides **53** and **27** paired to antiparallel (a) and parallel (b) DNA. Experimental conditions as in Table 3 (T = 1 °C).

of temperature on the CD spectral features of complex **38**:d$(A)_{10}$ (data not shown) was quite minute.

Hybridization Properties of Fully Modified Asymmetric F-OPA Oligoamides with Complementary DNA. To investigate the preferential strand alignment of (*Z*)-F-OPA in the duplex, the asymmetric oligomers **53** and **55** were synthesized and their hybridization properties to parallel and antiparallel DNA assessed and compared to the corresponding OPA and PNA sequences (Table 3).

All of the three OPA-oligoamides **51**−**53** lost the ability to bind to complementary antiparallel DNA, since no cooperative melting could be observed. However, all of the OPA oligoamides bound to parallel DNA. The (*Z*)-OPA isomer **52** thereby showed a slightly higher affinity than the (*E*)-isomer **51** (ΔT_m = +9.9 and +3.4 °C, respectively).[42,57] This stays in contrast to PNA, which prefers the antiparallel over the parallel binding mode. Again, the F-OPA oligomer **53** exhibited the lowest affinity in the OPA series (T_m ~ 6 °C). In all melting curves of **53** with DNA, a second transition at 35−45 °C occurred. A melting experiment with **53** alone revealed a cooperative transition with an increase of hyperchromicity by ca. 15% and a T_m of 42 °C. The observed transitions therefore indicate aggregation of **53** to a yet unidentified structure. A similar behavior was not found for the PNA and OPA single strands **27** and **51**. They only showed a noncooperative increase in hyperchromicity as is expected for single strand destacking. Thus, it appears that the increased self-complex formation in the F-OPA series relative to OPA is a consequence of the introduction of fluorine into the double bond.

It was surmised that a longer strand would lead to a complex of higher stability due to the additional base pairs and thus provide further insight into the pairing properties of the F-OPA system. However, and much to our surprise, no melting transition could be observed when F-OPA **55** was paired to antiparallel or parallel DNA. Instead of forming a stable hybrid with corresponding DNA, oligoamide **55** aggregated into a more stable self-complex with a transition at ca. 65 °C. This observation was confirmed by the presence of a similar transition in the UV melting curve of the single strand (see Supporting Information, Figure S33). On the basis of these observations, we assume that self-complex formation is efficiently competing with DNA binding.

The CD spectra of **53** paired to complementary antiparallel and parallel DNA confirmed the findings obtained by UV melting experiments (Figure 7). Indeed, the CD spectral features of the mixture **53**:**46** (Figure 7a) were radically different from those of the reference duplex **27**:**46** and reminiscent of the CD spectra of DNA single strands. On the other hand, the CD spectra of the complexes **53**:**47** and **27**:**47** were similar (Figure 7b) and reminiscent of those of B-type right-handed DNA duplexes, with positive bands around 260 and 220 nm and a negative band in the 250 nm region. Thus, the CD spectra confirmed duplex formation when **53** was paired to complementary parallel DNA **47** and showed no evidence for pairing to antiparallel DNA **46**.

(56) Efimov, V. A.; Choob, M. V.; Buryakova, A. A.; Kalinkina, A. L.; Chakhmakhcheva, O. G. *Nucleic Acids Res.* **1998**, *26*, 566−575.
(57) Gautschi, D.; Leumann, C. J. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1211−1213.

The changes in the CD spectral features of the mixture **53**:**46** with increasing temperature were minimal, unlike the case of the reference duplex **27**:**46** (see Supporting Information). On the other hand, all of the three bands of the CD spectrum of the parallel hybrid **53**:**47** were subjected to bathochromic shifts upon increasing the temperature. Furthermore, the decrease in intensity of the positive band at 220 nm with increasing temperature led to a sigmoidal pattern that is indicative for base-paired double strands.

Summary and Conclusions. In this work, we presented the synthesis of the F-OPA monomers containing the bases adenine and guanine starting from compound **4** in 16 and 14 steps, along similar lines as already reported for the thymine-containing monomeric unit **1**.[43] Single (*Z*)-adeninyl-F-OPA containing PNA oligoamides along with fully modified F-OPA sequences containing the bases A and T were synthesized by solid-phase chemistry. We found that ammonolytic deprotection was a major problem, which prevented the synthesis of fully deprotected, G-containing F-OPAs. Indeed, no conditions that allowed a neat removal of the isobutyryl protecting groups of the G bases together with cleavage of the oligoamides from the solid support, while oligoamide integrity was maintained, could be unraveled. The stability of the duplexes formed by the thymine and adenine F-OPA containing PNAs revealed to be highly dependent on the sequential position of the modification with T_m values ranging from +2.4 to −8.1 °C/modification compared to native PNA. This dependence was observed in the antiparallel and the parallel binding modes.

When analyzing the DNA-binding properties of fully modified F-OPAs, the question arising first is whether the OPA scaffold is fundamentally competent to bind to DNA. The T_m of PNAs containing OPA or F-OPA mutations, which show higher stability in some sequence contexts compared to pure PNA, as well as the observed binding of fully modified *E*- and *Z*-OPAs to DNA, are in favor of a competent pairing system. On the basis of this analysis we conclude that the chemical nature of the central amide unit in PNA is an important element determining its affinity to nucleic acids, its triplex formation behavior, and the preferred strand orientation in DNA/PNA duplexes. From the pairing properties of *E*,*Z*-OPA we further conclude that the geometric orientation of the base linker unit in PNA (*E* or *Z* rotameric form) is not a relevant structural parameter in determining affinity and preferred strand orientation upon duplex formation. This comes from the observation that both *E*- and *Z*-OPAs bind to complementary parallel DNA with similar T_m. Moreover, the studies with F-OPA here indicate that the dipolar properties of the amide functionality are also not a significant feature for binding orientation and strength. It thus seems that solvation effects determine the strong DNA affinity of PNA.

We found that the mixed-base F-OPA 15-mer **55** showed a high propensity for self-complex formation which can effectively compete for DNA binding. This feature seems to be less expressed in the nonfluorinated OPAs, indicating that dipolar effects may play an important role in the molecular association of these uncharged oligomers. Alternatively, self-aggregation could be due to the further enhanced hydrophobic nature of F-OPA relative to OPA. An increased tendency for self-aggregation is not an unexpected phenomenon for uncharged, hydrophobic DNA analogues.[58] This may be one of the reasons why nature chose a charged phosphodiester backbone for its genetic material.

Experimental Section

Monomer Syntheses. The synthesis and analytical data for compounds **10** and **11** were already described.[43]

2-[3-Benzyloxy-1-(2-benzyloxyethyl)propoxy]tetrahydropyran (5). To a solution of diol **4** (50 g, 0.24 mol) in dry THF (1200 mL) was carefully added NaH (32.04 g, 3 equiv) at 0 °C. The resulting white suspension was vigorously stirred at room temperature for 1 h. Benzyl bromide (108.9 g, 75 mL, 2.5 equiv) was then added and the mixture stirred overnight. The clear yellow solution was then cooled (0 °C) and quenched with water (500 mL). The aqueous layer was separated and extracted twice with *tert*-butyl methyl ether. The combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (hexane/*tert*-butyl methyl ether 6:1 → 4:1) to yield **5** as a yellow oil (82.1 g, 87%).

Data for **5**: R_f 0.63 (hexane/*tert*-butyl methyl ether 2:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41−1.44 (m, 4H), 1.68−1.70 (m, 1H), 3.36−3.41 (m, 1H), 3.46−3.51 (m, 2H), 3.58 (td, J = 1.9, 3.3, 2H), 3.78−3.83 (m, 1H), 3.93 (m, 1H), 4.38−4.48 (m, 4H), 4.53−4.55 (m, 1H), 7.20−7.28 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.2, 25.4, 31.1, 34.5, 35.6, 63.0, 66.7, 67.3, 72.2, 72.9, 72.9, 98.6, 127.4, 127.5, 127.6, 127.7, 128.3, 128.3, 138.4, 138.7; MS (EI$^+$) *m/z* 384 (3), 302 (3), 301 (18), 300 (31), 299 (61), 284 (9), 209 (17), 193 (43), 181 (69), 107 (66), 91 (100), 85 (96); EI-MSHR *m/z* calcd for $(C_{24}H_{32}O_4)^+$ 384.2300, found 384.2300 $(M + H)^+$.

1,5-Bis(benzyloxy)pentan-3-ol (6). A solution of compound **5** (82 g, 0.21 mol) in 1.4 L of absolute EtOH was treated with pyridinium tosylate (5.36 g, 0.1 equiv) and heated to 55 °C for 2.5 h. The reaction mixture was then cooled to 0 °C, treated cautiously with saturated $NaHCO_3$ (60 mL), and concentrated. The residue was dissolved in CH_2Cl_2 (300 mL) and washed once with water (300 mL). The aqueous layer was extracted twice with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4 and evaporated. Chromatographic separation (hexane/*tert*-butyl methyl ether 2:1 → *tert*-butyl methyl ether) gave pure alcohol **6** as a yellow oil (60.2 g, 94%).

Data for **6**: R_f 0.13 (hexane/*tert*-butyl methyl ether 2:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.76−1.83 (m, 4H), 3.62−3.71 (m, 4H), 3.99−4.07 (m, 1H), 4.53 (s, 4H), 7.29−7.36 (m, 10H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.6, 68.5, 69.4, 73.2, 127.7, 128.4, 138.1; MS (EI$^+$, EtOAc) *m/z* 301.24 $(M + H)^+$; HRMS (ESI$^+$) *m/z* calcd for $(C_{19}H_{25}O_3)^+$ 301.1803, found 301.1793 $(M + H)^+$.

1,5-Bis(benzyloxy)pentan-3-one (7). To a solution of compound **6** (60 g, 0.20 mol) in THF/DMSO 1:1 (800 mL) was added IBX (134.2 g, 2.4 equiv) and the mixture was stirred at room temperature for 4 h. The white suspension was cooled to 0 °C and diluted with saturated $NaHCO_3$ (130 mL). The mixture was then filtered over Celite and extracted twice with CH_2Cl_2. The combined organic layers were evaporated, and the residue was purified by chromatography (hexane/*tert*-butyl methyl ether 3:1) to yield ketone **7** (60.91 g, 100%) as a colorless oil.

Data for **7**: R_f 0.50 (hexane/*tert*-butyl methyl ether 1:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.70 (t, J = 6.3, 4H), 3.70 (t, J = 6.4, 4H), 4.46 (s, 4H), 7.21−7.25 (m, 10H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 43.4, 65.1, 73.2, 127.6, 127.7, 128.4, 138.1; MS (EI$^+$, EtOAc) *m/z* 280 (7), 208 (34), 207 (77), 192 (11), 174 (14), 159 (9), 146 (14), 107 (73), 101 (86), 91 (100), 73 (73); HRMS (ESI$^+$) *m/z* calcd for $C_{19}H_{22}O_3Na$ $(M + Na)^+$ 321.1466, found 321.1479 $(M + Na)^+$.

5-Benzyloxy-3-(2-benzyloxyethyl)-2-fluoropent-2-enoic Acid Ethyl Ester (8). To a suspension of NaH (8.85 g, 1.0

(58) Benner, S. A. *Acc. Chem. Res.* **2004**, *37*, 784−797.

equiv) in dry THF (1 L) was slowly added a solution of **7** (60.5 g, 0.2 mol) in THF (0.5 L) at −78 °C and the mixture stirred for 0.5 h at this temperature and then for 0.5 h at room temperature. After cooling again to −78 °C, a solution of diethyl fluorocarbethoxymethyl phosphonate (58.92 g, 1.2 equiv) in THF (0.6 L) was added and the cooling bath removed after 1 h. After 2 h, the mixture was quenched with water (600 mL) and EtOAc (200 mL). The aqueous layer was extracted with CH_2Cl_2 and EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (hexane/*tert*-butyl methyl ether 9:1) to yield ethyl ester **8** (53.83 g, 69%) as a colorless oil.

Data for **8**: R_f 0.69 (hexane/*tert*-butyl methyl ether 2:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.34 (t, 3H, J = 7.2), 2.64 (dt, 2H, J = 3.6, 6.9), 2.91 (dt, 2H, J = 1.4, 6.7), 3.60−3.65 (m, 4H), 4.27 (q, 2H, J = 7.1), 4.50 (s, 4H), 7.28−7.33 (m, 10 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.1, 30.8, 31.4, 61.2, 67.7, 68.8, 72.8, 72.8, 127.5, 127.5, 128.3, 128.4, 131.8 (d, J_{CF} = 12.2), 138.2, 138.3, 145.6 (d, J_{CF} = 251.6), 160.8 (d, J_{CF} = 34.8); ^{19}F NMR ($CDCl_3$) δ −125.0; MS (EI$^+$) *m/z* 387 (2), 385 (5), 356(5), 336 (3), 318 (2), 295 (45), 275 (30), 260 (24), 250 (16), 245 (28), 229 (33), 219 (27), 199 (51), 189 (69), 181 (60), 169 (52), 155 (41), 91 (100), 73 (43); HRMS (ESI$^+$) *m/z* calcd for $(C_{23}H_{28}O_4F)^+$ 387.1971, found 387.1979 $(M + H)^+$.

3-Fluoro-4-(2-hydroxyethyl)-5,6-dihydropyran-2-one (9). To a solution of ester **8** (53.8 g, 0.14 mol) in CH_2Cl_2 (1.5 L) was slowly added BCl_3 (350 mL, 1 M in CH_2Cl_2, 2 equiv). The reaction mixture instantly turned red and was stirred for 2 h at room temperature. The solution was then cooled (0 °C), and MeOH (120 mL) was added. The solvents were then removed and the residue was purified by column chromatography (gradient from 2.5 to 4% MeOH in CH_2Cl_2) to yield lactone **9** (21.2 g, 95%) as a slightly yellow oil.

Data for **9**: R_f 0.40 (MeOH/CH_2Cl_2 9:100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.60 (td, J = 0.8, 6.2, 2H), 2.67 (q, J = 5.9, 2H), 3.87 (t, J = 6.2, 2H), 4.41 (t, J = 6.2, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 27.3, 31.8, 59.5, 66.0, 133.2 (d, J_{CF} = 9.8), 143.4 (d, J_{CF} = 251.0), 159.6 (d, J_{CF} = 31.1); ^{19}F NMR ($CDCl_3$) δ −137.6 (t, J = 21.1); MS (EI$^+$) *m/z* 161 (3), 160 (8), 141 (20), 140 (100), 132 (24), 130 (93), 129 (87), 115 (38), 112 (43), 100 (26), 97 (26), 85 (57), 51 (70); HRMS (ESI$^+$) *m/z* calcd for $(C_7FH_9O_3)^+$ 160.0536, found 160.0534 $(M + H)^+$.

3-[2-(*tert*-Butyldimethylsilanyloxy)ethyl]-5-(6-chloropurin-9-yl)-4-fluoropent-3-en-1-ol (12). To a suspension of 6-chloropurine (5.0 g, 1.3 equiv), PPh_3 (9.18 g, 1.3 equiv), and DIAD (6.76 mL, 1.3 equiv) in dry THF (150 mL) was added a solution of diol **11** (7.5 g, 26.9 mmol) in dry THF (75 mL). After stirring the mixture for 12 h at room temperature, the solvent was removed and the residue purified by column chromatography (EtOAc/hexane 1:1 → 3:2) to yield alcohol **12** (3.96 g, 34%) as a white solid.

Data for **12**: R_f 0.27 (EtOAc/hexane 4:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.01 (s, 6H), 0.84 (s, 9H), 2.39 (td, J = 2.9, 6.2, 2H), 2.56 (t, J = 5.3, 2H), 3.16 (t, J = 5.7, 1H), 3.74 (t, J = 6.2), 3.85 (q, J = 5.4, 2H), 5.15 (d, J = 20.2, 2H), 8.30 (s, 1H), 8.76 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ −5.5, 18.2, 25.8, 31.9, 33.8, 41.7 (d, J_{CF} = 29.9), 60.0, 61.5, 118.4 (d, J_{CF} = 12.8), 131.6, 145.5, 149.7 (d, J_{CF} = 246.7), 151.3, 151.5, 151.9; ^{19}F NMR ($CDCl_3$) δ −113.9 (t, J = 20.4); MS (ES$^+$, CH_3CN:H_2O = 1:1 + 0.5% HCO_2H) *m/z* 415.21 $(M + H)^+$; HRMS *m/z* calcd for $(C_{18}ClFH_{29}N_4O_2Si)^+$ 415.1732, found 415.1732 $(M + H)^+$.

5-(6-Aminopurin-9-yl)-3-[2-(*tert*-butyldimethylsilanyloxy)ethyl]-4-fluoropent-3-en-1-ol (13). A solution of alcohol **12** (3.9 g, 9.4 mmol) in CH_2Cl_2 (20 mL) was transferred into an autoclave and degassed by an argon flow. A freshly prepared solution of saturated ammonia in MeOH (300 mL) was then added, the autoclave was closed and kept at 70 °C overnight. After cooling to room temperature, the solvents were removed, and the residue was purified by column chromatography (gradient of MeOH in CH_2Cl_2, 5% → 10%) to yield alcohol **13** (3.13 g, 84%) as a white solid.

Data for **13**: R_f 0.38 (CH_2Cl_2/MeOH 10:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.03 (s, 6H), 0.86 (s, 9H), 2.38 (td, J = 2.8, 6.4, 2H), 2.55 (t, J = 5.1, 2H), 3.72 (t, J = 6.4, 2H), 3.86 (t, J = 5.0, 2H), 5.05 (d, J = 19.8), 5.79 (s, 2H), 7.89 (s, 1H), 8.33 (s, 1H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ −5.4, 18.0, 25.9, 31.2, 32.9, 40.3 (d, J_{CF} = 16.5, C_5), 59.3, 60.8, 116.3 (d, J_{CF} = 12.8), 118.6, 140.7, 149.6, 151.2 (d, J_{CF} = 233.2), 152.8, 156.2; ^{19}F NMR (DMSO-d_6) δ −115.9 (t, J = 21.7); MS (EI$^+$) *m/z* 396 (4), 395 (12), 365 (7), 339 (37), 338 (100), 308 (23), 222 (21), 212 (59), 148 (32), 136 (96), 109 (43), 77 (42); HRMS (EI-MSHR) *m/z* calcd for $(C_{18}H_{30}N_5O_2FSi)^+$ 395.2153, found 395.2144 $(M + H)^+$.

9-{5-Azido-3-[2-(*tert*-butyldimethylsilanyloxy)ethyl]-2-fluoropent-2-enyl}-9*H*-purin-6-ylamine (14). To a cold (0 °C) solution of compound **13** (3.1 g, 7.84 mmol) in dry DMF (82 mL) were added PPh_3 (3.08 g, 1.5 equiv), LiN_3 (2.11 g, 5.5 equiv), and CBr_4 (3.90 g, 1.5 equiv). The resulting mixture was warmed to room temperature and stirred overnight. The solvent was removed and the residue was purified by column chromatography (gradient of MeOH in CH_2Cl_2 3% → 5%) to yield azide **14** (2.72 g, 82%) as a white solid.

Data for **14**: R_f 0.46 (CH_2Cl_2/MeOH 10:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ −0.02 (s, 6H), 0.82 (s, 9H), 2.35 (dt, J = 2.8, 6.4, 2H), 2.60 (t, J = 6.8, 2H), 3.50 (t, J = 6.8, 2H), 3.68 (t, J = 6.4, 2H), 5.01 (d, J = 21.8, 2H), 5.76 (s, 2H), 7.92 (s, 1H), 8.38 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ −5.6, 18.2, 25.8, 30.2, 31.1, 40.6 (d, J_{CF} = 30.5), 49.6, 61.3, 117.0 (d, J_{CF} = 14.0), 119.3, 140.2, 149.4, 151.3 (d, J_{CF} = 216.2), 153.2, 155.4; ^{19}F NMR ($CDCl_3$) δ −111.9 (t, J = 21.8); MS (EI$^+$) *m/z* 363 (6), 313 (9), 279 (17), 278 (66), 277 (100), 201 (41), 199 (62), 183 (43), 152 (41), 77 (52); HRMS (TOF-MS, MeOH/H_2O/HF) *m/z* cacld for $(C_{18}H_{30}N_8OFSi)^+$ 421.2295, found 421.2205 $(M + H)^+$.

***N*-(9-{5-Azido-3-[2-(*tert*-butyldimethylsilanyloxy)ethyl]-2-fluoropent-2-enyl}-9*H*-purin-6-yl)benzamide (15)**. To a cold (0 °C) solution of amine **14** (2.7 g, 6.4 mmol) in dry pyridine (90 mL) were added DMAP (0.23 g, 0.3 equiv) and benzoyl chloride (0.32 mL, 1.3 equiv), and the resulting solution was stirred at room temperature overnight. The reaction mixture was then cooled (0 °C) and quenched with water (10 mL) and concentrated NH_3 (15 mL). After leaving the solution at room temperature for 0.5 h, CH_2Cl_2 (150 mL) was added, and the organic layer was separated and washed with saturated $NaHCO_3$. The aqueous layer was extracted three times with CH_2Cl_2 (150 mL), and the combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (EtOAc) to yield **15** (2.63 g, 78%) as a slightly yellow oil.

Data for **15**: R_f 0.23 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ −0.02 (s, 6H), 0.83 (s, 9H), 2.37 (dt, J = 2.9, 6.4, 2H), 2.63 (t, J = 6.6, 2H), 3.53 (t, J = 6.7, 2H), 3.68 (t, J = 6.4, 2H), 5.11 (d, J = 21.6, 2H), 7.54 (t, J = 7.3, 2H), 7.63 (t, J = 7.3, 1H), 8.03 (d, J = 7.7, 2H), 8.15 (s, 1H), 8.83 (s, 1H), 8.96 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ −5.5, 18.2, 25.8, 30.3, 31.1, 40.8 (d, J_{CF} = 30.5), 49.6, 61.4, 117.5 (d, J_{CF} = 14.1), 122.6, 127.8, 128.9, 132.8, 133.6, 142.8, 149.4, 150.4 (d, J_{CF} = 213.1), 152.2, 155.6, 164.5; ^{19}F NMR ($CDCl_3$) δ −111.7 (t, J = 21.6); MS (EI$^+$) *m/z* 411 (2), 410 (8), 394 (9), 368 (12), 355 (7), 277 (5), 264 (24), 234 (23), 210 (18), 135 (100), 105 (100), 77 (90); HRMS (ESI$^+$) *m/z* calcd for $(C_{19}H_{19}N_8O_2FSi)^+$ 410.1615, found 410.1614 $(M + H)^+$.

***N*-{9-[5-Azido-2-fluoro-3-(2-hydroxyethyl)pent-2-enyl]-9*H*-purin-6-yl}benzamide (16)**. To a cooled (0 °C) solution of **15** (0.69 g, 1.3 mmol) in THF (20 mL) was added HF·pyridine (∼70% HF, 8 equiv, 0.27 mL). The resulting solution was stirred at room temperature overnight. The mixture was then quenched at 0 °C with saturated $NaHCO_3$ (4 mL) and water (40 mL) and extracted twice with CH_2Cl_2 (150 mL). The combined organic layers were dried over Na_2SO_4 and evaporated, and the residue was purified by column chromatography (CH_2Cl_2/MeOH 100:5) to yield alcohol **16** (0.51 g, 94%) as a white solid.

Data for **16**: R_f 0.12 (EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.44 (dt, J = 2.8, 6.3, 2H), 2.61 (t, J = 6.4, 2H), 3.56 (t, J = 6.6, 2H), 3.76 (t, J = 6.4, 2H), 5.14 (d, J = 21.3, 2H), 7.44−7.65 (m, 4H), 8.08 (d, J = 7.5, 1H), 8.20 (s, 1H), 8.82 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 30.0, 31.0, 41.1 (d, J_{CF} = 30.5), 49.5, 60.3, 117.1 (d, J_{CF} = 13.4), 122.3, 128.0, 128.82, 132.0, 132.9, 142.9, 149.4, 150.8 (d, J_{CF} = 247.9), 152.5, 164.5, 169.4; ^{19}F NMR ($CDCl_3$) δ −111.7 (t, J = 21.3); MS (ES$^+$, EtOAc) *m/z* 525.31 (M + H)$^+$, 547.35 (M + Na)$^+$; HRMS (ESI$^+$) *m/z* calcd for $C_{25}H_{33}N_8O_2FNaSi$ (M + Na)$^+$ 547.2377, found 547.2378 (M + Na)$^+$.

3-(2-Azidoethyl)-5-(6-benzoylaminopurin-9-yl)-4-fluoropent-3-enoic Acid (17). To a solution of compound **16** (0.5 g, 1.2 mmol) in CH_2Cl_2 (15 mL) was added at room temperature Dess−Martin periodinane (0.67 g, 1.3 equiv), and the white suspension was stirred for 2 h. The mixture was then poured into saturated $Na_2S_2O_3$ (15 mL) and saturated $NaHCO_3$ (30 mL) and allowed to stir for additional 10 min. After separation of the organic layer, the aqueous phase was extracted twice with CH_2Cl_2. The combined organic layers were washed once with brine, dried over Na_2SO_4, and evaporated. The thus obtained crude aldehyde was, without further purification, dissolved in *tert*-butyl alcohol (24 mL) and treated with 2-methyl-2-butene (6.1 mL, 45 equiv) followed by 10 mL of an aqueous solution of $NaClO_2$ (1.1 M) and NaH_2PO_4 (0.88 M). After stirring for 12 h at room temperature, the mixture was first concentrated and then treated with semiconcentrated $NaHCO_3$ (60 mL) and CH_2Cl_2 (60 mL). The organic layer was separated and extracted once with semiconcenctrated $NaHCO_3$ (100 mL), and the combined aqueous layers were acidified to pH 2.0 by addition of a 3 M solution of HCl. The resulting yellow solution was extracted five times with CH_2Cl_2 (150 mL). The combined organic layers were dried over Na_2SO_4 and evaporated, and the residue was purified by column chromatography (CH_2Cl_2/MeOH/AcOH = 100:4:0.5) to give acid **17** (0.32 g, 60%) as a slightly brownish solid.

Data for **17**: R_f 0.34 (100:5:0.5 CH_2Cl_2/MeOH/AcOH); ^{1}H NMR (DMSO, 300 MHz) δ 2.64 (t, J = 7.0, 2H), 3.12 (d, J = 2.8, 2H), 3.57 (t, J = 7.0, 2H), 5.27 (d, J = 21.3, 2H), 7.51−7.67 (m, 3H), 8.04 (d, J = 7.1, 2H), 8.46 (s, 1H), 8.75 (s, 1H), 11.18 (s, 1H); ^{13}C NMR (DMSO, 75.5 MHz) δ 29.6, 33.2, 45.4 (d, J_{CF} = 29.3), 48.9, 113.3 (d, J_{CF} = 14.0), 125.3, 128.7, 132.6, 133.6, 144.5, 150.5, 151.9, 152.0 (d, J_{CF} = 249.7), 152.4, 165.8, 171.6; ^{19}F NMR (DMSO) δ −111.5 (t, J = 21.3); MS (EI$^+$) *m/z* 426 (3), 425 (10), 395 (9), 351 (10), 321 (7), 303 (13), 239 (17), 211 (36), 135 (12), 122 (24), 105 (100), 77 (61); HRMS (ESI$^+$, H_2O/CH_3CN = 1/1) *m/z* calcd for $(C_{19}H_{18}N_8O_3F)^+$ 425.1485, found 425.1469 (M + H)$^+$.

5-(6-Benzoylaminopurin-9-yl)-4-fluoro-3-(2-{[(4-methoxyphenyl)diphenylmethyl]amino}ethyl)pent-3-enoic Acid (2). To a solution of acid **17** (0.3 g, 0.71 mmol) in dry pyridine (12 mL) was added PPh_3 (0.5 g, 2.75 equiv) and the resulting mixture was stirred at room temperature for 3 h. Concentrated NH_3 (1.5 mL) was then added and the solution stirred until a white precipitate was observed (∼3.5 h). The solvents were evaporated, and the residue was dried (10^{-2} Torr, 3 h). Water (50 mL) was added to the crude amine and the white suspension was kept under vigorous stirring for 0.5 h. The white precipitate was then filtered off and washed twice with water (50 mL). The aqueous layer was washed with toluene (100 mL), Et_2O (100 mL), and dichloromethane (100 mL). The combined organic layers were washed once with water (200 mL). The combined aqueous layers were then evaporated and dried (10^{-2} Torr, 2 h). The thus obtained crude amino acid was dissolved in dry DMSO (10 mL), treated with NEt_3 (0.5 mL, 5 equiv) and MMTrCl (0.44 g, 2.0 equiv), and stirred at room temperature for 12 h. Water and dichloromethane were then added. After separation of the phases, the aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated, and crude **2** was purified by column chromatography (CH_2Cl_2/MeOH/NEt_3 100:3:1 → 100:5:1). The resulting white solid was dissolved in CH_2Cl_2 (1.5 mL, previously filtered over basic Alox) and precipitated by addition into Et_2O (∼70 mL) to yield monomer **2** as a white powder (0.26 g, 54% over two steps).

Data for **2**: R_f 0.55 (CH_2Cl_2/MeOH/NEt_3 100:10:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.29 (t, J = 7.3, 9H), 2.37 (t, J = 5.8, 2H), 2.52 (t, J = 5.6, 2H), 3.01 (q, J = 7.3, 6H), 3.08 (d, J = 2.8, 2H), 3.73 (s, 3H), 5.18 (d, J = 21.3, 2H), 6.76 (d, J = 8.9, 2H), 7.13−7.26 (m, 12H), 7.29 (d, J = 7.3, 2H), 7.40−7.52 (m, 1H), 7.96 (d, J = 7.2, 2H), 8.29 (s, 1H), 8.69 (s, 1H), 9.48 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 8.5, 30.5, 34.6, 41.1 (d, J_{CF} = 27.4), 41.6, 45.7, 55.2, 113.2, 117.2 (d, J_{CF} = 9.3), 122.3, 126.4, 127.9, 128.1, 128.4, 128.7, 129.7, 132.7, 133.7, 137.6, 142.8, 145.7, 148.8, 151.2 (d, J_{CF} = 240.7), 151.8, 157.9, 165.0, 173.9; ^{19}F NMR ($CDCl_3$) δ −110.0 (t, J = 18.6); MS (ES$^+$, MeOH) *m/z* 671.42 (M + H)$^+$; HRMS (ESI$^+$-TOF, H_2O:CH_3CN = 1:1) *m/z* calcd for $(C_{39}H_{36}N_6O_4F)^+$ 671.2782, found 671.2756 (M + H)$^+$.

Diphenylcarbamic Acid 9-[5-(*tert*-Butyldimethylsilanyloxy)-2-fluoro-3-(2-hydroxyethyl)pent-2-enyl]-2-isobutyrylamino-9*H*-purin-6-yl Ester (18). To a solution of diol **11** (2.0 g, 7.2 mmol) in dry DMF (60 mL) was added O^6-diphenyl-carbamoyl-N^2-isobutyrylguanine[51] (3.59 g, 1.2 equiv) and the resulting white suspension was cooled to 0 °C. PPh_3 (2.45 g, 1.3 equiv) and DIAD (1.8 mL, 1.3 equiv) were added to the reaction mixture, which was stirred at room temperature overnight. After evaporation of the solvent, the crude product was purified by column chromatography (EtOAc/hexane 1:1 → 2:1) to yield alcohol **18** (2.83 g, 58%) as a slightly yellow oil.

Data for **18**: R_f 0.15 (CH_2Cl_2/MeOH 10:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.03 (s, 6H), 0.87 (s, 9H), 1.26, 1.29 (2s, 6H), 2.35 (dt, J = 2.7, 6.8, 2H), 2.58 (t, J = 6.3, 2H), 2.73 (sept, J = 6.8, 1H), 3.67−3.74 (m, 4H), 5.07 (d, J = 21.7, 2H), 7.25−7.34 (m, 2H), 7.34−7.46 (m, 8H), 8.07 (s, 1H), 8.12 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −5.4, 18.2, 19.2, 25.8, 31.3, 34.2, 36.7, 41.2 (d, J_{CF} = 30.9), 60.2, 61.3, 117.6 (d, J_{CF} = 12.4), 120.9, 126.9, 127.1, 129.2, 141.7, 144.4, 150.0 (d, J_{CF} = 244.0), 150.3, 151.7, 154.7, 156.1, 175.4; ^{19}F NMR ($CDCl_3$) δ −113.5 (t, J = 21.4); MS (EI$^+$) *m/z* 608 (13), 564 (4), 494 (3), 450 (3), 362 (2), 277 (100), 201 (30), 183 (18), 169 (100); HRMS (ESI$^+$, H_2O:CH_3CN = 1:1) *m/z* calcd for $(C_{35}H_{46}N_6O_5FSi)^+$ 677.3283, found 677.3303 (M + H)$^+$.

***N*-{9-[3-(2-Azidoethyl)-5-(*tert*-butyldimethylsilanyloxy)-2-fluoropent-2-enyl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl}-isobutyramide (19).** To a cooled (0 °C) solution of alcohol **18** (2.5 g, 3.7 mmol) in dry DMF (63 mL) were added PPh_3 (1.45 g, 1.5 equiv), LiN_3 (1.00 g, 5.5 equiv), and CBr_4 (4.90 g, 4 equiv). The resulting mixture was stirred at room temperature overnight. After quenching with MeOH (20 mL) and evaporation, the orange residue was dissolved in EtOAc (100 mL) and extracted with water (75 mL). The aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. Purification by column chromatography (CH_2Cl_2/MeOH 100:3) afforded **19** (1.33 g, 71%) as a slightly yellow solid.

Data for **19**: R_f 0.26 (EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.00 (s, 6H), 0.85 (s, 9H), 1.26, 1.28 (2s, 6H), 2.33 (dt, J = 2.8, 6.3, 2H), 2.63 (t, J = 7.2, 2H), 2.75 (sept, J = 6.9, 1H), 3.43 (t, J = 7.2, 2H), 3.66 (t, J = 6.2, 2H), 4.87 (d, J = 22.4, 2H), 7.88 (s, 1H), 9.25 (s, 1H), 12.13 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ −5.5, 18.2, 18.9, 25.8, 30.1, 30.7, 36.4, 40.8 (d, J_{CF} = 31.8), 49.8, 61.4, 117.3 (d, J_{CF} = 14.1), 120.2, 138.7, 147.9, 148.1, 150.8 (d, J_{CF} = 247.9), 155.3, 178.8; ^{19}F NMR ($CDCl_3$): δ −111.4 (t, J = 22.2); MS (ES$^+$, MeOH) *m/z* 507.36 (M + H)$^+$; HRMS (ESI$^+$-TOF, MeOH:H_2O:HFo = 70:25:5) *m/z* calcd for $(C_{22}H_{36}N_8O_3FSi)^+$ 507.2663, found 507.2650 (M + H)$^+$.

***N*-{9-[3-(2-Azidoethyl)-2-fluoro-5-hydroxypent-2-enyl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl}isobutyramide (20)**. To a cooled (0 °C) solution of **19** (1.3 g, 2.6 mmol) in dry THF (33 mL) was added HF·pyridine (∼70% HF, 0.45 mL, 8 equiv) and the reaction was allowed to proceed at room temperature for 12 h. After quenching with saturated $NaHCO_3$ (40 mL), the

mixture was extracted with CH_2Cl_2 (4 × 100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. Purification by column chromatography (CH_2Cl_2/MeOH 100:3 → 100:5) yielded alcohol **20** as a white solid (1.02 g, 100%).

Data for **20**: R_f = 0.22 (CH_2Cl_2/MeOH 10:1); ^{1}H NMR (MeOD, 300 MHz) δ 1.26, 1.28 (2s, 6H), 2.44 (td, J = 2.8, 6.8, 2H), 2.63 (t, J = 6.5, 2H), 2.77 (sept, J = 6.9, 1H), 3.56 (t, J = 6.5, 2H), 3.66 (t, J = 6.7, 2H), 5.09 (d, J = 21,1, 2H), 8.00 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 19.6, 31.0, 32.1, 37.2, 42.0 (d, J_{CF} = 30.5), 51.1, 61.2, 118.4 (d, J_{CF} = 14.0), 121.0, 141.4, 150.1, 151.0, 153.1 (d, J_{CF} = 247.3), 157.8, 182.1; ^{19}F NMR (MeOD) δ −114.4 (t, J = 21.1); MS (ES$^+$-MS, MeOH) m/z 414.99 (M + Na)$^+$, 392.94 (M + H)$^+$; HRMS (ESI$^+$-TOF) m/z calcd for $(C_{16}H_{22}N_8O_3F)^+$ 393.1798, found 393.1782 (M + H)$^+$.

3-(2-Azidoethyl)-4-fluoro-5-(2-isobutyrylamino-6-oxo-1,6-dihydropurin-9-yl)pent-3-enoic Acid (21). To a solution of alcohol **20** (0.95 g, 2.4 mmol) in a mixture of dry CH_2Cl_2 (30 mL) and DMSO (6 mL) was added Dess−Martin periodane[48] (1.33 g, 1.3 equiv) and the resulting white suspension stirred at room temperature for 3 h. The reaction mixture was then poured into a solution of saturated $Na_2S_2O_3$ (20 mL) and saturated $NaHCO_3$ (10 mL) and stirred for 10 min. After separation of the organic layer, the aqueous phase was extracted twice with CH_2Cl_2. The combined organic layers were washed once with brine, dried over Na_2SO_4, and evaporated. Without further purification, the crude aldehyde was dissolved in a mixture of *tert*-butyl alcohol (48 mL) and CH_2Cl_2 (5 mL), and 2-methyl-2-butene (12 mL, 45 equiv) was added followed by 20 mL of an aqueous solution containing $NaClO_2$ (1.1 M) and NaH_2PO_4 (0.88 M). After stirring for 12 h at room temperature and concentration in vacuo, semiconcentrated $NaHCO_3$ (100 mL) was added along with CH_2Cl_2 (100 mL). The organic layer was extracted once with semiconcentrated $NaHCO_3$, and the combined aqueous layers were acidified to pH 1.0 by addition of concentrated H_2SO_4. The resulting yellow aqueous solution was extracted five times with CH_2Cl_2 (250 mL), and the combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. Purification by column chromatography (CH_2Cl_2/MeOH/AcOH = 100:8:0.5) yielded acid **21** (0.40 g, 41%) as a white solid. Data for **21**: R_f 0.32 (CH_2Cl_2/MeOH/AcOH 100:10:0.5); ^{1}H NMR (MeOD, 300 MHz) δ 1.26, 1.28 (2s, 6H), 2.66 (t, J = 6.6, 2H), 2.80 (sep, J = 6.1, 1H), 3.25 (d, J = 3.0, 2H), 3.58 (t, J = 6.4, 2H), 5.12 (d, J = 21.1, 2H), 8.03 (s, 1H); ^{13}C NMR (MeOD, 75.5 MHz) δ 19.6, 31.3, 34.1, 37.3, 41.8 (d, J_{CF} = 30.5), 50.8, 115.3 (d, J_{CF} = 14.0), 121.0, 141.3, 150.1, 151.0, 153.9 (d, J_{CF} = 250.3), 157.8, 174.2, 182.1; ^{19}F NMR (MeOD): δ −111.8 (t, J = 20.98); MS (ES$^+$, MeOH) m/z 407.22 (M + H)$^+$; HRMS (ESI$^+$, MeOH/H_2O) m/z calcd for $(C_{16}H_{20}N_8O_4F)^+$ 407.1591, found 407.1600 (M + H)$^+$.

4-Fluoro-5-(2-isobutyrylamino-6-oxo-1,6-dihydropurin-9-yl)-3-(2-{[(4-methoxyphenyl)diphenylmethyl]amino}-ethyl)pent-3-enoic Acid (3). A solution of acid **21** (0.35 g, 0.86 mmol) in dry pyridine (15 mL) was treated with PPh_3 (0.62 g, 2.75 equiv) and stirred at room temperature for 3 h. Concentrated NH_3 (2.0 mL) was then added and the solution stirred until a white precipitate was observed (~3.5 h). The solvent was removed and the residue dried (10^{-2} Torr, 3 h). The mixture was suspended in water (50 mL) and the white precipitate filtered off and washed twice with water (50 mL). The aqueous solution was then washed consecutively with toluene (100 mL), Et_2O (100 mL), and CH_2Cl_2 (100 mL). The combined organic layers were washed once with water (200 mL). The combined aqueous layers were evaporated and dried (10^{-2} Torr, 2 h). The crude amine was then dissolved in dry DMSO (15 mL). After addition of NEt_3 (0.6 mL, 5 equiv) and MMTrCl (0.53 g, 2.0 equiv) and stirring for 12 h at room temperature, water and CH_2Cl_2 were added. The phases were separated, and the aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 evaporated and the crude product was purified by column chromatography (CH_2Cl_2/MeOH/NEt_3 100:5:1 → 100:10:1) to give a white solid, which was dissolved in dichloromethane (2.0 mL, previously filtered over basic Alox) and precipitated by addition into Et_2O (~70 mL) to give monomer **3** as a white powder (0.37 g, 65% over two steps). Data for **3**: R_f 0.40 (CH_2Cl_2/MeOH/NEt_3 100:10:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.13 (t, J = 7.3, 9H), 1.18, 1.21 (2s, 6H), 2.33 (t, J = 5.9, 2H), 2.48 (t, J = 5.5, 2H), 2.90 (q, J = 7.3, 6H), 2.94 (d, J = 2.4, 2H), 3.75 (s, 3H), 4.86 (d, J = 20.2, 2H), 6.77 (d, J = 8.9, 2H), 7.13−7.30 (m, 12H), 7.77 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.5, 18.9, 31.2, 35.4, 36.0, 40.8 (d, J_{CF} = 28.9), 41.8, 45.0, 55.2, 113.2, 116.8 (d, J_{CF} = 13.4), 120.6, 126.5, 127.9, 128.5, 129.8, 139.1, 145.7 147.9, 149.8 (d, J_{CF} = 239.7), 155.8, 158.04, 175.2, 179.3; ^{19}F NMR ($CDCl_3$) δ −112.7 (t, J = 16.0); HRMS (ESI$^+$-TOF, Cs^+, reserpine as standards) m/z calcd for $(C_{36}H_{37}N_6O_5FNa)^+$ (M + Na)$^+$ 675.2707, found 675.2733 (M + Na)$^+$.

Synthesis the Oligoamides. All oligoamides were synthesized on the 1.0 μmol scale on a Pharmacia LKB Gene Assembler Special DNA synthesizer as previously described[42,43] using the MMT/acyl strategy. The coupling efficiencies were in the range of 94−100% for all OPAs and >97% for unmodified PNAs. The glycine-containing solid support **22** was synthesized according to a known procedure.[42] Synthesis involved repetitive cycles, each comprising (i) deprotection of the *N*-protecting MMT-group with 3% trifluoroacetic acid (TFA) in dichloroethane (DCE), (ii) coupling of the free amine with the free carboxylic acid group of the incoming monomer (18 equiv) in the presence of HBTU (18 equiv) and the base DECA (36 equiv) in CH_3CN as the solvent using a coupling time of 20 min, and (iii) capping of the unreacted amino functionalities using isobutyric anhydride and 2,6-lutidine and *N*-methylimidazole as bases and THF as solvent. The deprotection of the *N*-MMT protecting group was monitored by the trityl assay.

Cleavage and Purification of Oligoamides. **Deprotection with Ammonia.** The resin was rapidly dried in an argon flux (2 min) and then introduced into an Eppendorf vial. One milliliter of concentrated ammonia was then added, and the mixture stirred for 2 min. The solution was heated to 55 °C for 12 h. The resin was then separated by filtration and the filtrate was washed with water (2 × 1 mL) and ethanol (2 × 1 mL). The resulting solution was dried and water (4 mL) was added.

Deprotection with Methylamine. The solid support was shortly dried and then introduced into an Eppendorf vial. One milliliter of methylamine (33% in ethanol) was then added and the resin was left at room temperature for 6 h with occasional stirring. The resin was then separated by filtration and the filtrate was washed with water (2 × 1 mL) and ethanol (2 × 1 mL). The resulting solution was dried and water (4 mL) was added.

After recording of the UV spectrum, the oligoamides were purified by reversed-phase HPLC and their identities were confirmed by ESI$^+$ mass spectrometry (see Table 1 in the Supporting Information).

UV Melting Experiments and CD Spectra. The concentration of the PNA oligoamides was calculated on the basis of the absorption at 260 nm, assuming the molar extinction coefficients of the nucleobases to be as in DNA, i.e., T, 8800 $M^{-1}\cdot cm^{-1}$; G, 11 700 and 15400 $M^{-1}\cdot cm^{-1}$, or based on values determined by Schütz et al.[42] UV melting curves were carried out on a Varian Cary 3E UV/vis spectrophotometer. Absorbances were monitored at 260 nm, and the heating rate was set to 0.5 °C/min. A heating−cooling−heating cycle in the temperature range 0−90 °C was applied. The absorbance melting curves were smoothed and the first-derivative curves obtained using the Varian WinUV software. For temperatures <20 °C, nitrogen was passed through the spectrophotometer to avoid condensation of water. To avoid evaporation of the solutions, 6−8 drops of dimethylpolysiloxane were added on top of the samples in the cell. All measurements were carried out in a buffer system consisting of 100 mM NaCl, 10 mM Na_2-

HPO_4 at pH 7.0 (adjusted with HCl (concentrated) and an aqueous solution of NaOH (15 M)). CD spectra were measured on a JASCO J-715 spectropolarimeter equipped with a temperature controller. The different experimental temperatures used are as indicated. Subsequently, the graphs were smoothed with a noise filter. All CD spectra are given in millidegrees from 210 to 320 nm.

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Supporting Information Available: General experimental methods; synthesis of oligoamides; ^{1}H NMR spectra of compounds **2**−**9** and **12**−**21**, as well as 2D ^{1}H/^{13}C HMBC of compounds **12** and **18** and ^{1}H NMR 2D T-ROESY of compound **12**; ^{1}H and ^{19}F NMR of dimer **24**; CD spectra of PNA **38**:d$(A)_{10}$ and PNA **43**:d$(A)_{15}$; temperature-dependent CD spectra of PNA **32**:DNA **46**, PNA **35**:DNA **46**, PNA **32**:DNA **47**, PNA **35**:DNA **47**, PNA **27**:DNA **46**, PNA **27**:DNA **47**, PNA **53**:DNA **46**, and PNA **53**:DNA **47**; UV melting curve of single strand **55**. This material is available free of charge via the Internet at http://pubs.acs.org.

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