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Pyrrolidino DNA with Bases Corresponding to the 2-Oxo Deletion Mutants of Thymine and Cytosine: Synthesis and Triplex-Forming Properties

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The dual recognition properties of pyrrolidino DNA species as parallel triplex-forming oligonucleotides were previously found to be strongly dependent upon the nature of the pyrimidine bases. In the structure–activity study presented here we were able to exclude this differential binding being due to their 2-oxo function. We had previously reported on the incorporation of pyrrolidino *C*-nucleosides into triplex-forming 2′-deoxyoligonucleotides (TFOs). The basic nitrogen atom that replaces the 4′-oxygen atom of the 2′-deoxysugar in such modified units introduces a positive charge in the third strand, and this is able to produce favourable electrostatic interaction with the negatively charged DNA target duplex. A first series of pyrrolidino pseudonucleosides with the bases isocytosine and uracil proved successful for GC base-pair recognition, but was unsuccessful for AT base-pair recognition within the parallel triplex binding motif. Here we report on the synthesis of the two novel 2′-deoxypyrrolidino nucleosides carrying the bases pyridin-2-one and 2-aminopyridine, their phosphoramidite building blocks and their incorporation into TFOs. Pyrrolidinylpyridin-2-one (dp2P) and -2-aminopyridine (dp2AP), prepared as part of a structure–activity profiling of pyrrolidino DNA in triplex binding, are deletion mutants of T and C, respectively. We found by T_m measurements that neither modification increased triplex binding efficiency relative to the iso-C- and -U-containing pyrrolidino TFOs. These experiments clearly show that the C4 carbonyl function, although important for triplex binding through indirect contributions in general, is not responsible for the differential binding of the latter two aminonucleosides and suggest that TFO conformation is more important.

Introduction

Stable and selective binding to genomic DNA by an oligonucleotide through triple-helix formation allows the flow of the genetic information in the cell to be modulated directly at the level of DNA. A triplex-forming oligonucleotide (TFO) binds to the exposed purine bases in the major groove of a DNA duplex either by *Hoogsteen* or by reverse-*Hoogsteen* base-pairing in the parallel or antiparallel triplex-binding motifs, respectively.[1] The so-called antigene strategy in theory confers complete control of gene expression and therefore constitutes an ideal method for gene therapy.[2–6] In reality, however, this strategy suffers from a number of significant limitations. Some are those encountered with most classical drugs, such as cellular uptake or in vivo stability, while others – such as pH dependence, restriction to homopurine target sites, and low thermal triplex stability, for instance – are specific to antigene agents. A variety of strategies to overcome these weaknesses and to improve the efficiency and applicability of antigene agents have been developed in the past.[7–9] The pH dependence, originating from the necessity of cytosine N-3 protonation, can be eliminated by replacement either with a more basic (and at physiological pH completely protonated) analogue[10–13] or with a charge-neutral analogue of cytosine.[14–18] Several approaches address the targeting of pyrimidine-purine inversion sites with high affinity. While reasonable solutions have been found for CG recognition,[19,20] no fully satisfactory solution for TA base pair recognition is so far available.[21–24]

Cuenoud and co-workers recently introduced the dual recognition concept to increase triplex stability.[25,26] Modified TFOs containing an aminoethoxy side-chain at C2′ of the ribose units were prepared in order to generate positive charges within the third strand. The target dsDNA can then be recognised not only through selective base–base interactions but also through additional, nonspecific salt bridges between these appropriately placed positive charges in the TFO and negatively charged backbone phosphate oxygen atoms.

We recently developed a similar dual recognition approach based on pyrrolidino nucleosides. Replacement of the 4′-oxygen atom in a deoxynucleoside of a TFO by a basic nitrogen atom, protonated at physiological pH, introduces an ideally located positive charge that can interact with a negatively charged pro-(*R*)-phosphate oxygen atom of the purine strand of a target dsDNA. Pyrrolidino pseudonucleosides containing the bases uracil (dpψU) or *N*-1-methyl uracil (dpψT) for AT base-pair recognition, together with the base isocytosine (dpψiC) for GC base-pair recogni-

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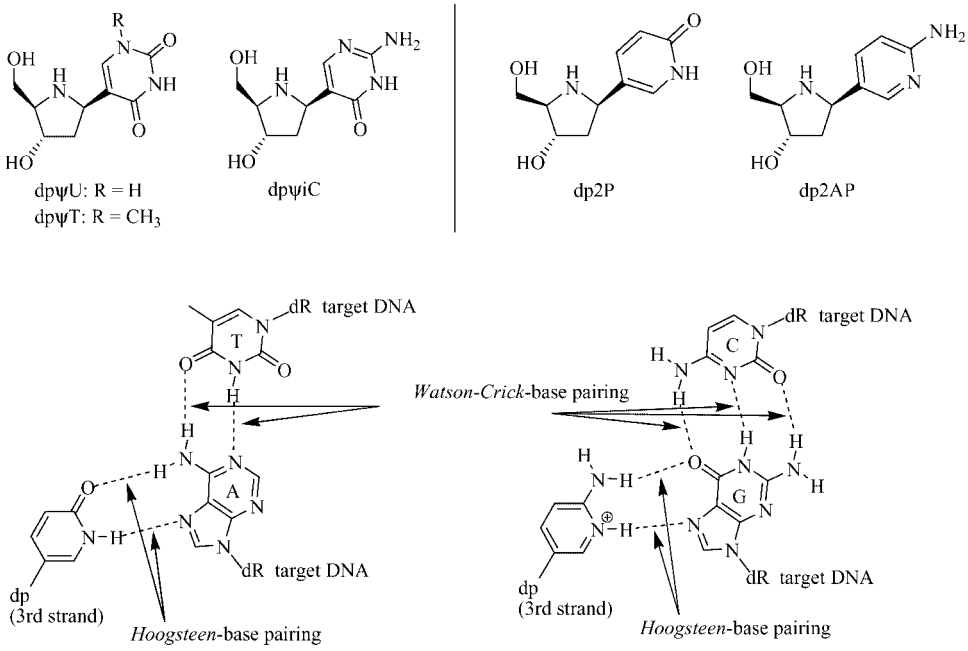


Figure 1. Top left: previously studied pyrrolidino pseudonucleoside analogues of deoxyuridine (dpψU), thymidine (dpψT) and deoxycytidine (dpψiC); top right: O4 deletion mutants dp2P and dp2AP of the corresponding nucleoside analogues; bottom: expected triplex pairing patterns.

tion within the parallel triplex binding motif, were explored (Figure 1). The dpψiC unit was demonstrated to be a successful substitute for cytidine in TFOs, resulting in increases in thermal triplex stability of about 2–2.5 °C per modification.[27] The TFOs containing the corresponding pyrrolidino nucleosides developed to replace T (dpψU and dpψT) instead showed target affinities inferior to those of the natural reference for an as yet unknown reason.[28]

To determine the effect of the C4-carbonyl function that is not directly involved in hydrogen bonding to the corresponding target purine base, pyrrolidino *C*-nucleosides bearing pyridin-2-one (dp2P) and 2-aminopyridine (dp2AP) units as nucleobases were designed. Pyridin-2-one represents the most simplified T analogue, keeping only the minimum of functional groups required for T–AT base triplet formation. 2-Aminopyridine had already previously been shown to be an excellent cytosine analogue in parallel triplexes in the deoxyribo backbone series.[10–13] In this article we report on the synthesis of the phosphoramidite building blocks of dp2P and dp2AP, their incorporation into TFOs as substitutes for T and C, respectively, and the evaluation of their triplex pairing properties. Finally we compare the results with those observed with the first generation of pyrrolidino pseudonucleosides.

Results and Discussion

Synthesis of Phosphoramidite Building Blocks

Of the different methods available for the preparation of *C*-nucleosides[29] and imino-sugar-based *C*-nucleosides,[30] our efforts first concentrated on palladium-catalysed Heck couplings of a pyrroline sugar analogue with the corresponding aglycons. This reaction had been previously been successfully applied for the synthesis of the first generation of pyrrolidino pseudonucleosides.[31] However, reactions between enamine **1** (Figure 2) and different halogenated derivatives of the required aglycons with use of various P- and As-ligands and different reaction conditions never produced the desired nucleosides. Alternative methods involving the addition of the organometallic derivative of the nucleobases variously to the corresponding lactam **2**,[32,33] to the corresponding hemiaminal **3** or to the *N*-acyliminium ion[34–38] obtained in situ from **3** proved unsuccessful as well.

A number of pyrrolidino *C*-nucleosides with aromatic (hetero)cycles, such as substituted phenyl groups, imidazoles or 9-deazapurines, as aglycons were synthesised by Schramm and co-workers as transition state inhibitors for

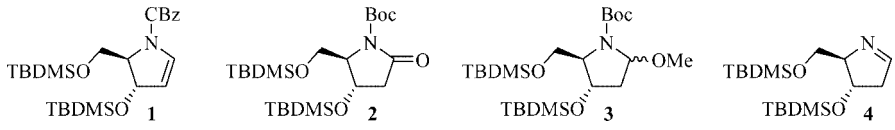
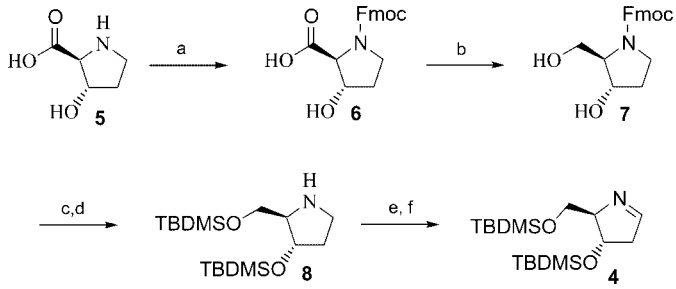


Figure 2. Chemical structures of the different *N*-glycosyl donors used in C-glycoside formation.

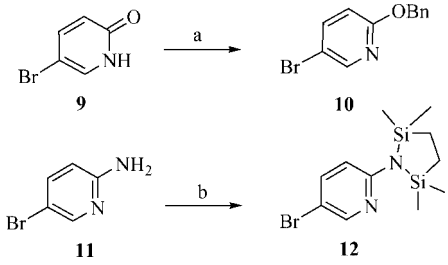


Scheme 1. Synthesis of imine **4**. (a) Fmoc-Cl, dioxane, 5% $NaHCO_3$ soln., 0 °C to room temp., 9 h, 98%; (b) BH_3·$(CH_3)_2S$, THF, reflux, 2 h, 97%; (c) TBDMS-Cl, imidazole, THF, room temp., 2 h; (d) piperidine, THF, room temp., 12 h, 90% over 2 steps; (e) NCS, hexane, room temp., 1 h; (f) LTMP, THF, –78 °C, 2 h, 74% over two steps.

nucleoside hydrolases or nucleoside phosphatases. The aglycon was introduced through addition of the corresponding aryllithium or aryl-Grignard reagents to the imine function of substituted 3,4-dihydro-2*H*-pyrroles.[39–42] A similar strategy with imine **4** as sugar component (Figure 2) finally proved also to be successful in the synthesis of dp2P and dp2AP.

The imine **4** was synthesised in six steps and 62% overall yield starting from *trans*-3-hydroxy-L-proline (Scheme 1). The amino function of the starting material was Fmoc-protected and the carboxylic acid was then selectively reduced to the primary alcohol to provide diol **7**. Both hydroxy groups were converted into TBDMS ethers and the pyrrolidino ring nitrogen atom was deprotected. Treatment of intermediate **8** with NCS in hexane resulted in the *N*-chloro analogue. Subsequent LTMP-mediated elimination of HCl at –78 °C by analogy to published procedures[39,40] afforded imine **4** in good yield. Although similar imines had been reported to decompose easily, **4** was isolated by column chromatography and proved to be stable at room temp.

The appropriately protected aglycons were prepared as shown in Scheme 2. 5-Bromo-2-hydroxypyridine (**9**) was selectively *O*-benzyl-protected to yield **10** by treatment with benzyl bromide and silver carbonate, whereas the 2-amino derivative **11** was converted into its silyl derivative **12**.

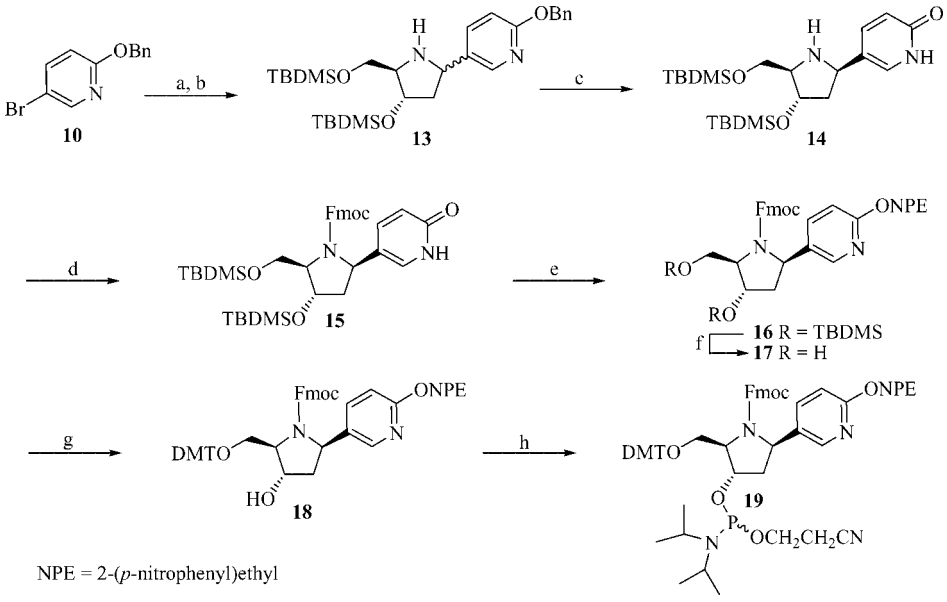


Scheme 2. Synthesis of the aglycons **10** and **12**. (a) BnBr, Ag_2CO_3, THF, reflux, 5 h, 93%; (b) butyllithium, $Cl(Me)_2SiCH_2CH_2Si(Me)_2Cl$, THF, –78 °C, 2 h30, 79%.

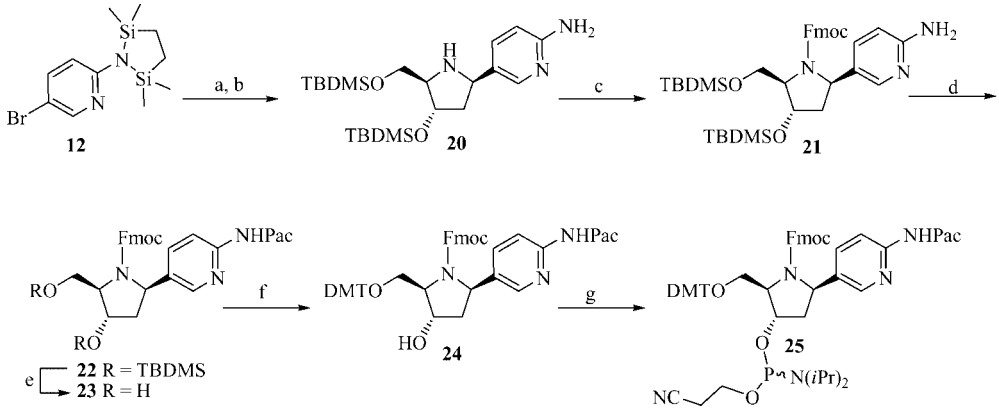
Treatment of benzyl-protected pyridone **10** with butyllithium and subsequent addition of imine **4** to the obtained lithio derivative at –78 °C resulted in the formation of a 1:10 α/β mixture of the desired product **13** in 50% yield (Scheme 3). The separation of the two anomers presented some difficulties at this stage and was conducted only after the next step. The benzyl protecting group was removed by catalytic hydrogenolysis and the pure β anomer of **14** was isolated in 35% yield over two steps. The configuration at the pseudo-anomeric centre was determined by ^{1}H NMR-NOE experiments: a strong mutual NOE was observed between 2-H and 5-H of the pyrrolidine ring, establishing the β configuration.

The Fmoc group had previously been shown to be compatible with oligonucleotide synthesis and was therefore used to protect the pyrrolidine nitrogen atom. Bevers et al. reported the necessity of protecting the pyridin-2-one oxygen atom in oligonucleotide synthesis without giving further details.[43] In preliminary experiments we also experienced side reactions between the unprotected pyridone moiety and the incoming phosphoramidite building block during oligonucleotide synthesis, dramatically decreasing the efficiency of the synthesis. Therefore, protection of the pyridone seemed to be required, and the 2-(*p*-nitrophenyl)ethyl group (NPE) was chosen for this purpose. The NPE group was incorporated through the Mitsunobu reaction. Although all the starting material was consumed and only one new product seemed to be formed, the fully protected compound **16** was obtained only in a rather low yield of 35%. The silyl ethers were cleaved off with acidic methanol to afford the diol **17** in 67% yield. Standard tritylation of the primary alcohol (→ **18**), followed by phosphitylation, finally afforded phosphoramidite building block **19**.

A similar route was applied for the synthesis of dp2AP and its phosphoramidite derivative (Scheme 4). The stabase adduct of 2-amino-5-bromopyridine (**12**) was subjected to bromo–lithium exchange followed by treatment with imine **4** to furnish the β-pyrrolidino pseudonucleoside **20** as determined after purification by ^{1}H NMR-NOE experiments (see Experimental Section). The stabase protective group was hydrolysed during column chromatography. Fmoc protection was performed selectively at the pyrrolidine nitrogen atom. For the exocyclic amino group of 2-aminopyridine, classical protective groups suitable for oligonucleotide synthesis (acetyl, benzoyl, etc) had been reported to exhibit increased stability against ammonolysis and not to be removable under classical oligonucleotide deprotection conditions.[11] In order to avoid resorting to harsher deprotection conditions, the more labile phenoxyacetyl (Pac) group was incorporated on the primary amine and intermediate **22**



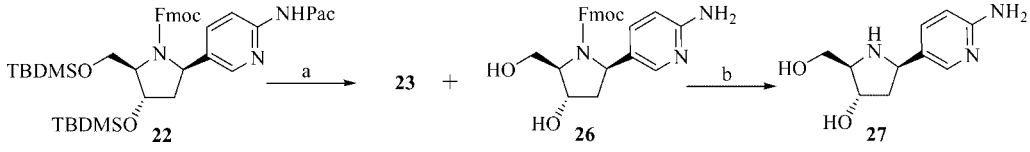
Scheme 3. Synthesis of phosphoramidite **19**. (a) Butyllithium, THF, –78 °C, 1 h; (b) **4**, THF, –78 °C; (c) 10% Pd/C, H_2, MeOH, room temp., 30 min., 35% over two steps; (d) Fmoc-OSu, THF, dioxane, 1 M aq. $NaHCO_3$, room temp., 1 h 30, 80%; (e) PPh_3, DIAD, *p*-nitrophenylethanol, dioxane, room temp., 8 h, 35%; (f) 1 M aq. HCl/MeOH 1:10, room temp., 24 h, 67%; (g) DMTCl, pyridine, room temp., 5 h, 74%; (h) CEPCl, *i*Pr_2NEt, THF, room temp., 2 h, 82%.



Scheme 4. Synthesis of phosphoramidite **25**. (a) Butyllithium, THF, –78 °C, 1 h; (b) **4**, THF, –78 °C, 2 h, 19%; (c) Fmoc-OSu, THF, dioxane, 1 M aq. $NaHCO_3$, room temp., 30 min, 75%; (d) Pac_20, pyridine, room temp., 2 h, 99%; (e) 1 M aq. HCl/MeOH 1:10, room temp., 24 h, 99%; (f) DMTCl, pyridine, room temp., 2 h, 88%; (g) CEPCl, *i*Pr_2NEt, THF, room temp., 1 h30, 90%.

was formed in excellent yield. The diol **23** was obtained quantitatively from **22** by acidic methanolysis of the silyl ethers. DMT protection afforded compound **24**, and introduction of the phosphoramidite moiety yielded the building block **25**.

Pac-deprotected product **26**, formed on extended exposure of compound **22** to acidic conditions, was isolated and converted into the fully deprotected dp2AP (**27**) by treatment with piperidine (Scheme 5). Pyrrolidino *C*-nucleoside **27** was fully characterised and the β configuration at the anomeric centre was confirmed by ^{1}H NMR-NOE experiments, indicating that no anomerisation had occurred during any step of the synthesis, in particular during the TBDMS deprotection step performed under strongly acidic conditions (see Experimental Section). The phosphoramidite building blocks of dp2P and dp2AP (**19** and **25**, respec-



Scheme 5. Synthesis of dp2AP. (a) 1 M aq. HCl/MeOH 1:10, room temp., 30 h, 76% of **23** and 23% of **26**; (b) piperidine, DMF, room temp., 12 h, 91%.

tively) were then incorporated into oligonucleotides by automated solid-phase oligonucleotide synthesis and their triplex-forming affinities were evaluated.

Synthesis of Oligodeoxynucleotides

Oligodeoxynucleotides were synthesised by standard solid-phase phosphoramidite chemistry with a DNA synthesiser. Minor modifications to the synthesis cycle were introduced for the incorporation of the unnatural building blocks. More precisely, the coupling time was extended from 1.5 to 6 min and the standard activator tetrazole was replaced by the more powerful (*S*-benzylthio)-1*H*-tetrazole. For the sequences containing dp2AP, the classical acetyl capping step was replaced by a phenoxyacetyl (Pac) capping step. Coupling efficiencies for the modified units were typically >97%, according to trityl assay. Standard ammonia deprotection resulted in cleavage of the crude oligonucleotides from the solid support and removal of all protecting groups (including Fmoc). For all sequences containing NPE protective groups, a two-step deprotection procedure first involving treatment with 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) in acetonitrile (1 M) at 60 °C for 8 h, followed by ammonolysis, was applied. Use of a weaker base (DBU, 0.5 M in pyridine or 1 M in acetonitrile), lower temperatures or shorter reaction times resulted in incomplete NPE removal. Isomerisation at the anomeric centre by elimination/addition of the pyrrolidine ring nitrogen atom under these conditions could be excluded on the basis of an NMR experiment with the monomer **17**.

Crude oligomers were purified by ion-exchange HPLC and characterised by ESI$^-$ mass spectrometry. A summary of the modified oligomers synthesised and their mass spectrometric analysis is provided in Table 1. The triplex-forming properties of the modified oligodeoxynucleotides were subsequently examined by UV melting curve analysis.

Pairing Properties of dp2P-Containing TFOs with DNA Duplexes in the Parallel Motif

The melting temperatures (T_ms) for TFO dissociation from a 21-mer dsDNA target are summarised in Table 2. Relative to the natural third strand **Ref1**, TFO **dp1** displayed a significant loss in triplex stability of about 11 °C per modification (Entry 4). This corresponds to a further destabilisation relative to dpψU- and even dpψT-containing triplexes (Entries 2 and 3, respectively).

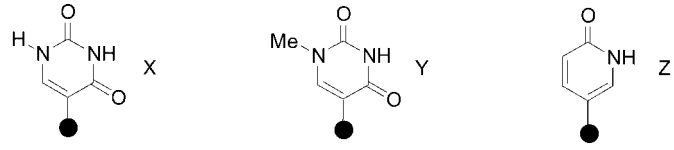
This result was confirmed by circular dichroism (CD) spectroscopy. The CD spectra of the triplexes were measured at different temperatures below and above the third strand melting temperature. The resulting curves exhibited almost identical shapes except in the region below 235 nm, with a maximal change in the CD spectral properties at 220 nm (Figure 3 left). Consequently, the variation of the CD intensity at a fixed wavelength of 220 nm was monitored between 10 and 50 °C and a sigmoidal curve was obtained (Figure 3, right). The first derivative of this curve gave a T_m value of 32 °C, which is in perfect agreement with the one determined by UV-melting experiments.

To determine target binding selectivity, another target duplex (Target 2, Table 3) exposing a guanine instead of an adenine opposite to the pyrrolidine analogue was prepared. The data obtained from melting curve experiments are summarised in Table 3, together with the T_m values measured with the matched duplex (Target 1) for comparison. The unmodified TFO **Ref1** used as reference gave a strongly destabilised triplex with a T_m of about 30 °C and a ΔT_m of –12.6 relative to Target 1, as expected for a mismatch situa-

Table 1. TFO sequences and ESI-MS data for the oligonucleotides investigated in this study.

TFO	Sequence	Mod.	[M – H]$^-$ (found)	[M – H]$^-$ (calcd)
dp1	5′-d(TTTTXCTCTCTCTCT)	dp2P	4393.0	4392.9
dp2	5′-d(TTTTTCTXTCTCTCT)	dp2AP	4407.1	4406.9
dp3	5′-d(TTTTTXTCTXTCTCT)	dp2AP	4389.5	4388.9
dp4	5′-d(TTTTTXTXTXTXTXT)	dp2AP	4334.8	4335.0
dp5	5′-d(TTTXXXXTTTTXTTT)	dp2AP	4335.0	4335.0

Table 2. Sequence of TFOs and T_m data (°C) for third strand dissociation from UV melting curves (260 nm).[a]

Base = 

Entry	TFO	Sequence	T_m[b]	ΔT_m per modification.
1	**Ref1**	5′-d(TTTTTCTCTCTCTCT)	43.1	0
2	**Ref2**	5′-d(TTTT**X**CTCTCTCTCT)	38.4	–4.7
3	**Ref3**	5′-d(TTTT**Y**CTCTCTCTCT)	37.0	–6.1
4	**dp1**	5′-d(TTTT**Z**CTCTCTCTCT)	32.0	–11.1

[a] Target duplex = d(GCTAAAAAGAGAGAGAGATCG)·d(CGATCTCTCTCTCTTTTTAGC), T_m = 57.0 ± 1.0 °C. [b] Single strand concentration = 1.2 μM in 140 mM KCl, 7 mM NaH_2PO_4, 0.5 mM $MgCl_2$, pH 6.0.

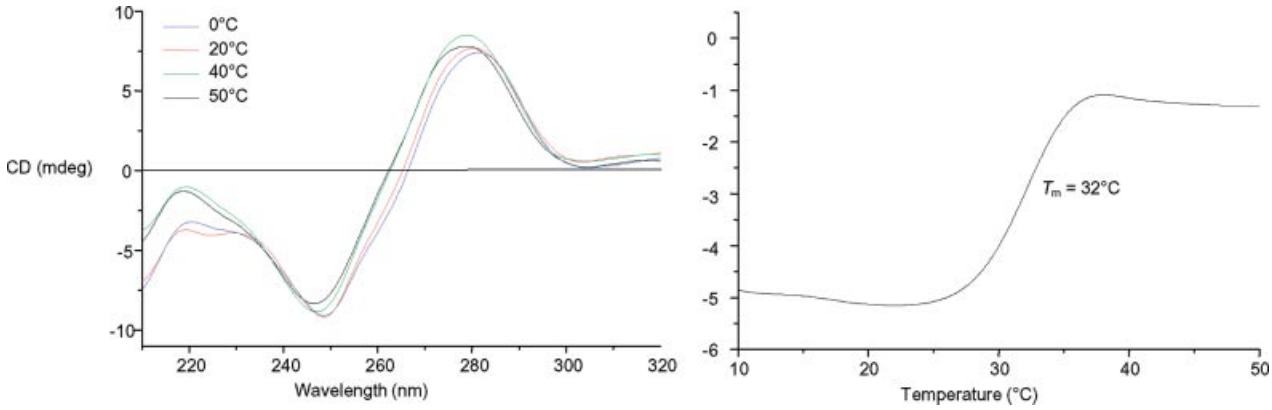


Figure 3. CD spectra of the triplex-containing TFO **dp1** (Table 4); left: spectra measured at different temperatures; right: temperature scan from 10 to 50 °C at 220 nm; single strand concentration = 1.2 μM in 140 mM KCl, 7 mM NaH_2PO_4, 0.5 mM $MgCl_2$, pH 6.0.

Table 3. Sequence of TFOs and T_m data (°C) for third strand dissociation from UV melting curves (260 nm).

Target 1	3′-d(CGATTTTTCTCTCTCTCTAGC) *5′-d(GCTAAAAAGAGAGAGAGATCG)*		Target 2	3′-d(CGATTTTCCTCTCTCTCTAGC) *5′-d(GCTAAAAGGAGAGAGAGATCG)*	
TFO	5′-d(TTTTXCTCTCTCTCT)		TFO	5′-d(TTTTXCTCTCTCTCT)	
Entry	TFO	X	T_m[a](Target 1)	T_m[a](Target 2)	ΔT_m
1	Ref1	T	43.1	30.5	–12.6
2	Ref2	dpψU	38.4	30.5	–7.9
3	dp1	dp2P	32.0	29.5	–2.5

[a] Single strand concentration = 1.2 μM in 140 mM KCl, 7 mM NaH_2PO_4, 0.5 mM $MgCl_2$, pH 6.0. T_m of target duplex 1 = 57.0 ± 1.0 °C. T_m of target duplex 2 = 61.0 ± 1.0 °C.

tion (Entry 1). The dpψU-containing TFO **Ref2** and the duplex Target 2 formed a triplex that was also substantially less stable than that with the duplex Target 1 (Entry 2). The T_m value was in the same range as for the natural triplex, indicating that this system presented a mismatch as well. Not much difference in thermal stability between match and mismatch was encountered in the case of the triplex with **dp1** (Entry 3). The pyrrolidinylpyridin-2-one unit thus seems to recognise the AT base pair within the parallel binding motif with very low affinity.

The missing C4-carbonyl oxygen atom thus seems to play an important role in triplex stability. It may positively influence the stacking interactions and/or the solvation properties. From the experiments presented here, however, it can be ruled out that this oxygen atom may be responsible for the loss in affinity of a dpψU (or dpψT) residue relative to an unmodified T as reported previously.

Pairing Properties of dp2AP-Containing TFOs with DNA Duplexes in the Parallel Motif

At pH 6.0, all the modified TFOs (Table 4) gave triple helical structures exhibiting diminished stability relative to the natural system. However, the destabilisation decreases with an increased number of modifications, from –5 °C per modification in the case of monomodified TFO **dp2** to –3 °C per modification for TFO **dp4** containing five modifications (Entries 2 and 6, respectively). In a different sequence context, runs of four contiguous dp2AP units led to further destabilisation (**dp5**, Entry 9). This phenomenon is well known for cytosines: the relative stabilities of triple helices containing C^+-GC triplets drop as the number of adjacent C^+-GC triplets increases, due to a further decrease in the pK_a value of cytosine N3 and local repulsion of contiguous positive charges in the pile of protonated cytosines.[44] However, these composition effects have been reported to be less pronounced for 2-aminopyridine *C*-nucleosides.[11] It appears to be verified in our case, since the additional loss in stability is low.

More interesting is the pH variation study of the dp2AP unit. Triplexes containing TFOs **dp2** and **dp3** were strongly affected by the pH value: an increase in pH from 6.0 to 7.0 resulted in a significant drop in T_m (Entries 2–3 and 4–5), which was consistent with the presence of pH-dependent functionalities in the TFO (cytosine and 2-aminopyridine bases, as well as pyrrolidine units). However, the pH dependence seemed to arise mainly from the cytosine, since the replacement of a natural C with a dp2AP unit (**dp2** → **dp3**) resulted in a lower T_m decrease when the pH was increased from 6.0 to 7.0. This suggestion was confirmed with the TFOs **dp4** and **dp5**, with all natural cytosines replaced by dp2AP moieties. These produced triple helical structures that showed no drop in T_m over the same pH range, despite the five pyrrolidinyl-2-aminopyridine units (Entries 6–7 and 13–14). Only at pH > 8 were no triplexes formed any more (Entries 8–9 and 15–16). Although the dp2AP gave less stable triplexes than the references at pH 6.0, the weaker pH dependence provided an advantage at pH 7.0. TFO **dp5**, for instance, exhibited a loss in stability of 4.3 °C per modification at pH 6.0, but at pH 7.0 it formed a triplex with a

Table 4. Sequence of TFOs and T_m data (°C) for third strand dissociation from UV melting curves (260 nm).[a]

Entry	TFO	Sequence	Target duplex	pH	T_m[b]	ΔTm per modification
1	Ref1	5′-d(TTTTTCTCTCTCTCT)	1	6.0	43.1	0
2	dp2	5′-d(TTTTTCTXTCTCTCT)	1	6.0	38.0	–5.1
3				7.0	19.1	–
4	dp3	5′-d(TTTTTXTCTXTCTCT)	1	6.0	36.1	–3.5
5				7.0	22.1	–
6	dp4	5′-d(TTTTTXTXTXTXTXT)	1	6.0	26.9	–3.2
7				7.0	27.0	–
8				8.0	22.1	–
9				9.0	n.d.[c]	–
10	Ref5	5′-d(TTTCCCCTTTTCTTT)	2	6.0	27.2	0
11				7.0	n.d.[c]	0
12				8.0	n.d.[c]	0
13	dp5	5′-d(TTTXXXXTTTTXTTT)	2	6.0	5.7	–4.3
14				7.0	7.0	–
15				8.0	n.d.[c]	–
16				9.0	n.d.[c]	–

[a] C = 5-methylcytosine, X = dp2AP; Target duplex 1 = d(GCTAAAAAGAGAGAGATCG)·d(CGATCTCTCTCTCTTTTTAGC), T_m = 57.0 ± 1.0 °C; Target duplex 2 = d(GCTAAAGGGGAAAAGAAATCG)·d(CGATTTCTTTTCCCCTTTAGC), T_m = 60.0 °C ± 1.0 °C. [b] Single strand concentration = 1.2 μM in 140 mM KCl, 7 mM NaH_2PO_4, 0.5 mM $MgCl_2$, pH 6.0. [c] No T_m detectable.

similar T_m, whereas the reference **Ref5** showed no detectable affinity for the target duplex (Entries 10–11 and 13–14).

Incorporation of dp2AP into TFOs gives rise to triplexes displaying astonishingly low pH dependence in the pH 6–8 range, relative to corresponding systems containing cytidines, methylcytidines or any pyrrolidino nucleosides studied so far. Both the pyrrolidine and the pyridine nitrogen atoms seem to benefit from a high protonation state up to pH 8, generating triplexes that would not suffer from pH effects under physiological conditions. This remarkable property is compromised, however, by the triplex destabilisation properties induced by dp2AP. The missing carbonyl group might also play a role in these negative effects here for the same reasons as given for dp2P.

Conclusions

Pyrrolidino *C*-nucleosides have the potential to be excellent candidates for the dual recognition approach in DNA duplex recognition as constituents of parallel TFOs. However, positive evidence is so far limited to pyrrolidino pseudoiso-C as a nucleobase, forming a stable dpψiC-GC triplet. In order to promote pyrrolidino DNA as an antigene agent the critical parameters for effective and general dual recognition still need to be identified and tuned appropriately.

Of the multitude of factors that can be modified to increase the binding efficiency of pyrrolidino DNA, that of base variation was examined in this study. More specifically, pyrrolidinylpyridin-2-one (dp2P) and -2-aminopyridine (dp2AP), both deletion mutants – of dpψU and dpψiC, respectively – were developed as tools to test the importance of the C4-carbonyl oxygen atom in triplex affinity. We found a general tendency towards decreased triplex stability in both cases. The obtained results show that this functional group plays an important, general, but indirect, role in triplex recognition, possibly through favourable stacking interactions and/or solvation effects.

More importantly, these results clearly show that the C4-carbonyl function is not responsible for the differential stabilities of dpψiC-GC and dpψU/T-AT base triplets. Since the dual recognition motif strongly relies on electrostatic interactions between an amino and a phosphate function, and since such interactions are strongly distance-dependent, it seems more likely that local structural differences between the dpψiC-GC and the dpψU-AT (or dpψT-AT) triplets, or of the triple helical conformation in general, could result in variations in the N-O(P) distances that are important enough to result in the loss of the favourable electrostatic contact in the latter case. Pyrrolidine-modified TFOs with an RNA backbone, which would be expected to give triple helices of the A conformation based on the known 3′-*endo* conformation of the nucleosides, might therefore shed light on the influence of conformational parameters on triplex stability. Corresponding experiments are currently underway in our laboratory.

Experimental Section

General: Reactions were carried out under Ar in anhydrous solvents. Solvents for extractions were technical grade and were distilled prior to use. Reagents were purchased from Fluka AG, Aldrich and Merck (highest quality available). NMR spectra were measured with a Bruker AC 300 or a Bruker DRX 400 spectrometer. ^{1}H NMR spectra were recorded at 300 or 400 MHz. Chemical shifts are reported in ppm relative to the residual undeuterated NMR solvent ($CHCl_3$: δ = 7.27 ppm, [D_5]DMSO: δ = 2.50 ppm, CD_2HOH: δ = 3.35 ppm, HDO: δ = 4.8 ppm). Coupling constants *J* are in Hz. ^{13}C NMR spectra were recorded at 75 or 100 MHz. Chemical shifts are reported in ppm relative to the residual undeuterated NMR solvent ($CHCl_3$: δ = 77.00 ppm, [D_5]DMSO: δ = 39.70 ppm, CD_2HOD: δ = 49.30 ppm). ^{31}P NMR spectra were recorded at 162 MHz. Chemical shifts are reported in ppm relative to 85% H_3PO_4 as an external standard. ^{1}H NMR difference-NOE experiments were recorded at 400 MHz. Electron impact (EI) mass

spectra were recorded with an AutoSpeq Q VG instrument with an ionisation energy of 70 eV. Fast atom bombardment (FAB^+) mass spectra were also recorded with an AutoSpeq Q VG. Electrospray ionisation (ESI) mass spectra were recorded either with a Fisons Instrument VG Platform (oligonucleotides) or with an Applied Biosystems, Sciex QSTAR Pulsar (monomers). TLC was performed with precoated silica gel plates (SIL G-25 UV_{254}) from Macherey–Nagel. Visualisation was achieved by use of UV light (254 nm) or staining solutions (cerium reagent, anisaldehyde reagent, ninhydrin reagent). Flash chromatography (FC) was performed with silica gel 60 (particle size 40–63 μm) from Fluka. The compounds based on C-nucleosides dp2P and dp2AP were named as substituted pyrrolidines and numbered as described below (Figure 4). ^1H and ^{13}C NMR spectroscopic data for compounds **15**–**19** and **21**–**26**, bearing Fmoc groups, were not reported, due to broad signals from coalescence (carbamate bond).

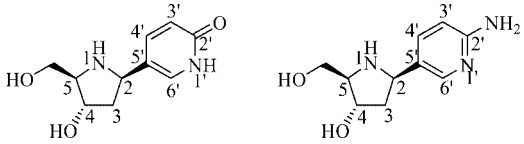


Figure 4. Numbering scheme for pyrrolidino pseudonucleosides **13**–**19** derived from dp2P and **20**–**27** derived from dp2AP used for NMR assignments.

***trans*-*N*-[(9-Fluorenylmethoxy)carbonyl]-3-hydroxy-L-proline (6):** A solution of 9-fluorenylmethyl chloroformate (11.0 g, 42.5 mmol) in dioxane (150 mL) was added at 0 °C to a suspension of *trans*-3-hydroxy-L-proline (**5**, 5.5 g, 41.9 mmol) in dioxane (300 mL) and $NaHCO_3$ (5%, 150 mL). After 2 h, the cooling bath was removed and stirring was continued for 7 h. Most of the dioxane was evaporated, and the aq. layer was washed twice with Et_2O, acidified to pH 3.0 with aq. HCl (2 N) and extracted with AcOEt (3×). The combined org. layers were dried ($MgSO_4$) and concentrated to give 14.7 g (98%) of **6** as a white powder. ^1H NMR (300 MHz, [D_6]-DMSO, 25 °C): δ = 1.80–1.98 (m, 2 H, 4-H), 3.48–3.54 (m, 2 H, 5-H), 4.05–4.37 (m, 5 H, 2-H, 3-H, Fmoc-H, Fmoc-CH_2), 5.52, 5.59 (2× br s, 1 H, OH), 7.29–7.44 (m, 4 H, Fmoc-H), 7.62–7.69 (m, 2 H, Fmoc-H), 7.88–7.91 (m, 2 H, Fmoc-H), 12.91 (br s, 1 H, COOH) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO, 25 °C): δ = 34.38, 35.37 (2× t, C-4), 47.33, 47.97 (2× t, C-5), 49.61 (d, Fmoc-C), 69.70, 69.98 (2× t, Fmoc-C), 70.91, 71.18 (2× d, C-3), 75.81, 76.91 (2× d, C-2), 123.15 (d, Fmoc-C), 128.14, 128.24 (2× d, Fmoc-C), 130.16 (d, Fmoc-C), 130.71 (d, Fmoc-C), 143.66, 143.75 (2× s, Fmoc-C), 146.69, 146.82 (2× s, Fmoc-C), 157.03, 157.21 (2× s, -NCO_2-), 174.79, 175.09 (2× s, COOH) ppm. MS (FAB^+): *m*/*z* (%) = 354 (26) [M + H]$^+$, 338 (24), 309 (100). HR-MS (FAB^+, [M + H]$^+$): 354.13574 ($C_{20}H_{20}NO_5$ requires: 354.13415).

(2*R*,3*S*)-*N*-[(9-Fluorenylmethoxy)carbonyl]-3-hydroxy-2-(hydroxymethyl)pyrrolidine (7): $BH_3{\cdot}(CH_3)_2S$ (2 M in THF, 96.2 mL, 192.4 mmol) was added to a solution of **6** (13.0 g, 36.8 mmol) in dry THF (130 mL). The solution was heated at reflux for 2 h and cooled to room temp., and the reaction was quenched by addition of MeOH. The solution was concentrated, the residue was dissolved in AcOEt, washed with H_2O and dried ($MgSO_4$), and the solvents were evaporated. FC (toluene/THF 1:1 to 5:6) gave 12.1 g (97%) of **7** as a white solid. TLC (toluene/THF 1:1): R_f 0.24. ^1H NMR (300 MHz, [D_6]DMSO, 25 °C): δ = 1.66–1.75 (m, 1 H, 4-H), 1.94–2.07 (m, 1 H, 4-H), 3.08–3.20 (m, 1 H, CH_2-OH), 3.28–3.57 (m, 4 H, 2-H, 5-H, CH_2-OH), 4.17–4.21 (m, 1 H, 3-H), 4.27 (s, 3 H, Fmoc-H, Fmoc-H), 4.76–4.82 (m, 1 H, *H*O-CH_2), 4.92–4.94 (m, 1 H, HO-C-3), 7.33 (t, $J_{H,H}$ = 7.2 Hz, 2 H, Fmoc-H), 7.42 (t, $J_{H,H}$ = 7.0 Hz, 2 H, Fmoc-H), 7.66 (d, $J_{H,H}$ = 7.4 Hz, 2 H, Fmoc-H), 7.90 (d, $J_{H,H}$ = 7.4 Hz, 2 H, Fmoc-H) ppm. MS (FAB^+): *m*/*z* (%) = 340 (100, [M + H]$^+$), 309 (22), 179 (60), 155 (52), 119 (83). HR-MS (FAB^+, [M + H]$^+$): 340.15485 ($C_{20}H_{22}NO_4$ requires: 340.15488).

(2*R*,3*S*)-3-{[(*tert*-Butyl)dimethylsilyl]oxy}-2-{[(*tert*-butyl)dimethylsilyl]oxymethyl}pyrrolidine (8): *tert*-Butyldimethylsilyl chloride (11.7 g, 77.6 mmol) was added to a solution of **7** (12.0 g, 35.4 mmol) and imidazole (8.4 g, 123 mmol) in dry THF (150 mL). After 2 h, piperidine (23 mL, 233 mmol) was added and stirring was continued for 12 h. The solution was concentrated and the residue was distributed between AcOEt and H_2O. The org. layer was dried ($MgSO_4$) and the solvents were evaporated. FC (CH_2Cl_2/MeOH/25% aq. NH_3 50:1:1 to 25:1:1) afforded 11.0 g (31.8 mmol, 90%) of **8** as a yellow oil. ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.06 (s, 12 H, 4× CH_3-Si), 0.88, 0.89 (2× s, 18 H, 2× $(CH_3)_3$C-Si), 1.63–1.72 (m, 1 H, 4-H), 1.83–1.95 (m, 1 H, 4-H), 2.22 (s, 1 H, NH), 2.90–3.11 (m, 3 H, 2-H, 5-H), 3.54–3.64 (m, 2 H, CH_2-OTBDMS), 4.10–4.15 (m, 1 H, 3-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = –5.50, –5.45, –4.76, –4.59 (4× q, 4× CH_3-Si), 18.02, 18.27 (2× s, 2× $(CH_3)_3C$-Si), 25.82, 25.90 (2× q, 2× $(CH_3)_3$-C-Si), 35.28 (t, C-4), 44.82 (t, C-5), 63.12 (t, CH_2-OTBDMS), 68.14 (d, C-2), 73.77 (d, C-3) ppm. MS (FAB^+): *m*/*z* (%) = 346 (100, [M + H]$^+$), 288 (8), 200 (17). HR-MS (FAB^+, [M + H]$^+$): 346.25998 ($C_{17}H_{40}NO_2Si_2$ requires: 346.25976).

(4*S*,5*R*)-4-{[(*tert*-Butyl)dimethylsilyl]oxy}-5-{[(*tert*-butyl)dimethylsilyl]oxymethyl}-1-azacyclopent-1-ene (4): *N*-Chlorosuccinimide (500 mg, 3.74 mmol) was added to a solution of **8** (1.0 g, 2.89 mmol) in hexane (40 mL). After 1 h, the suspension was filtered through Celite and the solvents were evaporated. The residue was dissolved in dry THF (50 mL) and cooled to –78 °C. A solution of tetramethylpiperidine (1.18 mL, 6.97 mmol) in THF (30 mL), previously treated with BuLi (1.6 M in hexane, 4.06 mL, 6.50 mmol) at 0 °C and stirred at 0 °C for 30 min, was added to the solution at –78 °C over 1 h until no more *N*-chloro compound was detected by TLC. After 1 h, the mixture was warmed to room temp., most of the THF was evaporated, and the residue was distributed between *tert*-butyl methyl ether and H_2O. The org. layer was dried ($MgSO_4$) and the solvents were evaporated. FC (hexane/AcOEt 5:1 to 4:1) gave 730 mg (74%, 2.12 mmol) of **4** as a yellow oil. TLC (hexane/AcOEt 4:1): R_f 0.34. ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.02, 0.04, 0.08 (3× s, 12 H, 4× CH_3-Si), 0.87, 0.88 (2× s, 18 H, 2× $(CH_3)_3$C-Si), 2.44 (d, $J_{H,H}$ = 18.4 Hz, 1 H, 3-H), 2.75 (dd, $J_{H,H}$ = 6.2, 18.4 Hz, 1 H, 3-H), 3.56 (dd, $J_{H,H}$ = 4.8, 10.3 Hz, 1 H, CH_2-OTBDMS), 3.86 (dd, $J_{H,H}$ = 3.3, 10.3 Hz, 1 H, CH_2-OTBDMS), 4.06 (m, 1 H, 4-H), 4.37 (d, 1 H, $J_{H,H}$ = 6.6 Hz, 5-H), 7.61 (s, 1 H, 2-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = –5.55, –5.44, –4.72, –4.64 (4× q, 4× CH_3-Si), 18.01, 18.25 (2× s, 2× $(CH_3)_3C$-Si), 25.79, 25.85 (2× q, 2× $(CH_3)_3$C-Si), 47.39 (t, C(3)), 63.08 (t, CH_2-OTBDMS), 71.12 (d, C-4), 83.47 (d, C-5), 165.95 (d, C-2) ppm. MS (FAB^+): *m*/*z* (%) = 344 (100, [M + H]$^+$), 154 (10), 115 (17).

2-Benzyloxy-5-bromopyridine (10): BnBr (4.1 mL, 34.5 mmol) and Ag_2CO_3 (4.75 g, 17.2 mmol) were added to a solution of **9** (5.00 g, 28.7 mmol) in dry THF (250 mL). The mixture was heated at reflux in the dark for 5 h and was then filtered through Celite and washed with diethyl ether, and the solvents were evaporated. The residue was purified by FC (AcOEt 2–5% in hexane) to yield 7.04 g (93%) of **10** as a white solid. TLC (AcOEt/hexane 2:98): R_f 0.26. ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 5.35 (s, 2 H, CH_2-Bn), 6.73 (d, $J_{H,H}$ = 8.7 Hz, 1 H, 3-H), 7.29–7.46 (m, 5 H, Bn-H), 7.66 (dd, $J_{H,H}$ =

2.5, 8.7 Hz, 1 H, 4-H), 8.21 (d, $J_{H,H}$ = 2.5 Hz, 1 H, 6-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 67.98 (Bn-CH_2), 111.89 (C5), 112.93 (C3), 127.97, 128.00, 128.49, 136.89 (Bn-C), 141.18 (C4), 147.44 (C6), 162.40 (C2) ppm. MS (EI$^+$): *m/z* (%) = 263/265 (56/56, [M]$^+$), 184 (8), 157/159 (15/15), 91 (100), 65 (55). HR-MS (EI$^+$, [M]$^+$): 262.9941 ($C_{12}H_{10}BrNO$ requires: 262.9946).

5-Bromo-2-(2,2,5,5-tetramethyl-1,2,5-azadisilolidin-1-yl)pyridine (12): A solution of 2-amino-5-bromopyridine (**11**, 5.00 g, 28.9 mmol) in dry THF (80 mL) was treated at –78 °C with *n*BuLi (1.6 M in hexane, 18.1 mL, 28.9 mmol). After 1 h at –78 °C, a solution of 1,2-bis(chlorodimethylsilyl)ethane (6.22 g, 28.9 mmol) in THF (15 mL) was added dropwise. After another 90 min at –78 °C, *n*BuLi (1.6 M in hexane, 18.1 mL, 28.9 mmol) was added, and the mixture was allowed to reach room temp. and stirred for an additional 2 h. Ice-cold brine (50 mL) was added and the mixture was quickly extracted with Et_2O (2 × 200 mL). The combined org. phases were dried ($MgSO_4$) and concentrated. Kugelrohr distillation (110–120 °C/0.1 mbar) gave 7.20 g (79%) of the stabase adduct **12** as a white solid. TLC (AcOEt/hexane 1:99): R_f 0.8. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.30 (s, 12 H, 4 × CH_3-Si), 0.82 (s, 4 H, 2 × CH_2-Si), 6.46 (dd, $J_{H,H}$ = 0.8, 8.9 Hz, 1 H, 3-H), 7.46 (dd, $J_{H,H}$ = 2.6, 8.9 Hz, 1 H, 4-H), 8.14 (dd, $J_{H,H}$ = 0.8, 2.6 Hz, 1 H, 6-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = –0.58 (4 × CH_3-Si), 8.54 (2 × *C*H_2-Si), 108.52 (C-5), 113.26 (C-3), 139.29 (C-4), 148.67 (C-6), 159.43 (C-2) ppm.

2-(2-Benzyloxypyridin-5-yl)-4-(*tert*-butyldimethylsilanyloxy)-5-(*tert*-butyldimethylsilanyloxymethyl)pyrrolidine (13): *n*BuLi (1.6 M in hexane, 4.49 mL, 7.2 mmol) was added dropwise at –78 °C to a stirred solution of **10** (1.90 g, 7.2 mmol) in dry THF (33 mL). After 1 h, a solution of **4** (823 mg, 2.4 mmol) in dry THF (12 mL) was slowly added and the mixture was stirred for 2 h. The reaction was quenched at –78 °C with water (40 mL) and the layers were separated. The aq. phase was extracted with ether (40 mL). The combined org. phases were dried ($MgSO_4$) and concentrated to yield a yellow oil. FC (AcOEt/hexane 1:8 to 1:5) afforded 809 mg of an α/β mixture of **13** as a yellow oil, which was used for the next step without further purification. A pure fraction of the β anomer was isolated for analytical characterisation. TLC (AcOEt/hexane 1:7): R_f 0.27. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.06, 0.07, 0.08 (3 × s, 12 H, 4 × CH_3-Si), 0.90, 0.91 (2 × s, 18 H, 2 × $(CH_3)_3$C), 1.73–1.90 (m, 2 H, 3-Hβ, NH), 1.97–2.04 (m, 1 H, 3-Hα), 3.14–3.18 (m, 1 H, 5-H), 3.55–3.69 (m, 2 H, CH_2O), 4.23–4.27 (m, 1 H, 4-H), 4.42 (dd, $J_{H,H}$ = 6.4, 9.7 Hz, 1 H, 2-H), 5.36 (s, 2 H, CH_2-Bn), 6.77 (d, $J_{H,H}$ = 8.8 Hz, 1 H, 3′-H), 7.30–7.47 (m, 5 H, Bn-H), 7.62 (dd, $J_{H,H}$ = 2.6, 8.8 Hz, 1 H, 4′-H), 8.12 (d, $J_{H,H}$ = 2.6 Hz, 1 H, 6′-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = –5.39, –4.69, –4.57 (4 × CH_3-Si), 18.05, 18.33 [2 × $(CH_3)_3$*C*-Si], 25.86, 25.94 [2 × (*C*H_3)$_3$C-Si], 43.78 (C3), 57.64 (C2), 64.81 (CH_2O), 67.57 (CH_2-Bn), 68.86 (C-5), 74.26 (C4), 111.01 (C-3′), 127.74, 127.91, 128.42 (5 C-Bn), 132.66 (C-5′), 137.40 (C-4′), 137.45 (C-Bn), 144.97 (C-6′), 162.93 (C-2′) ppm. Difference-NOE (400 MHz, $CDCl_3$, 25 °C): δ = 1.73–1.90 (H3β) → 1.97–2.04 (6.5%, H3α), 3.55–3.69 (2.6%, CH_2O), 4.23–4.27 (6.8%, H4), 7.62 (3.5%, 4′-H), 8.12 (2.6%, 6′-H); 1.97–2.04 (3-Hα) → 1.73–1.90 (19.1%, 3-Hβ), 3.55–3.69 (2.1%, CH_2O), 4.23–4.27 (3.1%, 4-H), 4.42 (5.1%, 2-H), 7.62 (2.0%, 4′-H), 8.12 (2.1%, 6′-H); 3.14–3.18 (5-H) → 3.55–3.69 (5.1%, CH_2O), 4.23–4.27 (2.8%, 4-H), 4.42 (3.4%, 2-H); 3.55–3.69 (CH_2O) → 3.14–3.18 (5.1%, 5-H), 4.23–4.27 (5.7%, 4-H); 4.23–4.27 (4-H) → 1.73–1.90 (6.0%, 3-Hβ), 1.97–2.04 (1.4%, 3-Hα), 3.14–3.18 (2.4%, 5-H), 3.55–3.69 (2.8%, CH_2O); 4.42 (2-H) → 1.97–2.04 (5.7%, 3-Hα), 3.14–3.18 (3.1%, 5-H), 7.62 (2.2%, 4′-H), 8.12 (7.9%, 6′-H); 6.77 (3′-H) → 5.36 (1.1%, CH_2-Bn), 7.62 (10.1%, 4′-H); 7.62 (4′-H) → 6.77 (14.2%, 3′-H); 8.12 (6′-H) → 4.42 (5.3%, 2-H), 5.36 (1.0%, CH_2-Bn). MS (ESI$^+$): *m/z* (%) = 1058 (34), 530 (100, [M + H]$^+$), 344 (8). HR-MS (ESI$^+$, [M + H]$^+$): 529.3275 ($C_{29}H_{49}N_2O_3Si_2$ requires: 529.3281).

4-(*tert*-Butyldimethylsilanyloxy)-5-(*tert*-butyldimethylsilanyloxymethyl)-2-(2-oxopyridin-5-yl)pyrrolidine (14): Pd/C (10% w/w, 81 mg) was added under argon to a solution of **13** (α/β mixture, 809 mg, 1.5 mmol) in MeOH (50 mL) and the black suspension was stirred under hydrogen. The reaction was monitored by TLC in AcOEt/hexane 1:4, stained with ninhydrin. As soon as no starting material was detectable any more, typically after 30–45 min, the palladium catalyst was filtered off over Celite and washed with MeOH, and the filtrate was concentrated and coevaporated with AcOEt to yield a greenish foam. FC (AcOEt/THF 1:0 to 4:1) afforded 352 mg of **14** (35% over two steps) as a yellowish foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.43. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.05, 0.06, 0.07, 0.07 (4 × s, 12 H, 4 × CH_3-Si), 0.89 (2 × s, 18 H, 2 × $(CH_3)_3$C), 1.62–1.69 (m, 1 H, 3-Hβ), 1.93 (ddd, $J_{H,H}$ = 1.7, 4.7, 9.6 Hz, 1 H, 3-Hα), 3.13–3.16 (m, 1 H, H5), 3.47 (dd, $J_{H,H}$ = 4.9, 7.6 Hz, 1 H, CH_2O), 3.59 (dd, $J_{H,H}$ = 3.9, 7.6 Hz, 1 H, CH_2O), 4.18–4.21 (m, 1 H, 4-H), 4.26 (dd, $J_{H,H}$ = 4.8, 7.4 Hz, 1 H, 2-H), 6.55 (d, $J_{H,H}$ = 7.1 Hz, 1 H, 3′-H), 7.37 (d, $J_{H,H}$ = 1.9 Hz, 1 H, 6′-H), 7.51 (dd, $J_{H,H}$ = 1.9, 7.1 Hz, 1 H, 4′-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = –5.39, –5.37, –4.69, –4.61 (4 × CH_3-Si), 18.02, 18.34 (2 × $(CH_3)_3$*C*-Si), 25.83, 25.95 (2 × (*C*H_3)$_3$-C-Si), 42.88 (C-3), 56.91 (C-2), 65.38 (CH_2O), 68.53 (C-5), 74.19 (C4), 120.10 (C-3′), 123.22 (C-5′), 131.49 (C-6′), 141.36 (C-4′), 165.05 (C-2′) ppm. Difference-NOE (400 MHz, $CDCl_3$, 25 °C): δ = 1.62–1.69 (3-Hβ) → 1.93 (7.3%, 3-Hα), 3.47 (1.5%, CH_2O), 4.18–4.21 (5.6%; 4-H), 4.26 (1.8%, 2-H), 7.37 (3.0%, 6′-H), 7.51 (3.6%, 4′-H); 1.93 (3-Hα) → 1.62–1.69 (7.6%, 3-Hβ), 4.18–4.21 (1.7%, 4-H), 4.26 (3.8%, 2-H), 7.37 (2.9%, 6′-H), 7.51 (1.0%, 4′-H); 3.13–3.16 (5-H) → 3.47 and 3.59 (5.7%, CH_2O), 4.18–4.21 (2.9%, 4-H), 4.26 (2.5%, 2-H); 3.47 (CH_2O) → 3.59 (19.9%, CH_2O), 4.18–4.21 (2.6%, 4-H); 3.59 (CH_2O) → 3.13–3.16 (4.1%, 5-H), 3.47 (10.7%, CH_2O), 4.18–4.21 (2.3%, 4-H); 4.18–4.21 (4-H) → 1.62–1.69 (3.1%, 3-H3), 1.93 (1.6%, 3-Hα), 3.13–3.16 (2.3%, 5-H), 3.47 and 3.59 (2.4%, CH_2O); 4.26 (2-H) → 1.93 (2.8%, 3-Hα), 3.13–3.16 (2.0%, 5-H), 7.37 (3.1%, 6′-H′), 7.51 (2.8%, 4′-H); 6.55 (3′-H) → 7.51 (4.5%, 4′-H); 7.37 (6′-H) → 1.62–1.69 (1.2%, 3-Hβ), 4.26 (3.3%, 2-H); 7.51 (4′-H) → 1.62–1.69 (2.5%, 3-Hβ), 4.26 (2.6%, 2-H), 6.55 (9.1%, 3′-H). MS (ESI$^+$): *m/z* (%) = 878 (6), 439 (100, [M + H]$^+$), 346 (5). HR-MS (ESI$^+$, [M + H]$^+$): 439.2819 ($C_{22}H_{43}N_2O_3Si_2$ requires: 439.2812).

Fluoren-9-ylmethyl 4-(*tert*-Butyldimethylsilanyloxy)-5-(*tert*-butyldimethylsilanyloxymethyl)-2-(2-oxopyridin-5-yl)pyrrolidine-1-carboxylate (15): A solution of Fmoc-OSu (541 mg, 1.6 mmol) in THF (8.5 mL) was added to a suspension of **14** (352 mg, 0.8 mmol) in dioxane (8.5 mL) and aq. $NaHCO_3$ (1 M, 8.5 mL). When no starting material was detected any more by TLC (1.5 to 2 h), 60 mL of aq. sat. NaCl was added and the product was extracted with 3 × 30 mL AcOEt. The org. layers were dried with $MgSO_4$ and concentrated to yield a yellow foam. The product was used for the next step without further purification. A pure sample of **15** was obtained as white foam after FC (AcOEt) for characterisation. TLC (THF): R_f 0.63. MS (ESI$^+$): *m/z* (%) = 1322 (20), 661 (100, [M + H]$^+$), 509 (4). HR-MS (ESI$^+$, [M + H]$^+$): 661.3488 ($C_{37}H_{53}N_2O_5Si_2$ requires: 661.3493).

Fluoren-9-ylmethyl 4-(*tert*-Butyldimethylsilanyloxy)-5-(*tert*-butyldimethylsilanyloxymethyl)-2-{2-[2-(4-nitrophenyl)ethoxy]pyridin-5-yl}pyrrolidine-1-carboxylate (16): Ph_3P (408 mg, 1.6 mmol) and (*p*-nitrophenyl)ethanol (260 mg, 1.6 mmol) were added under argon to a solution of **15** (278 mg, 0.4 mmol) in dry dioxane (4 mL). DIAD

(302 μL, 1.6 mmol) was then added dropwise to the stirred solution. After 8 h, the reaction mixture was concentrated and the yellow oily residue was purified by FC (AcOEt/hexane 2:3 to 1:1) to yield 118 mg (35%) of **16** as a yellow foam. TLC (AcOEt): R_f 0.41. MS (ESI$^+$): *m*/*z* (%) = 1621 (18), 1089 (59), 810 (87, [*M* + H]$^+$), 658 (100), 279 (81). HR-MS (ESI$^+$, [M + H]$^+$): 810.3963 ($C_{45}H_{60}N_3O_7Si_2$ requires: 810.3969).

Fluoren-9-ylmethyl 4-Hydroxy-5-(hydroxymethyl)-2-{2-[2-(4-nitrophenyl)ethoxy]pyridin-5-yl}pyrrolidine-1-carboxylate (17): A solution of aq. HCl (1 M, 256 μL) was added to a solution of **16** (151 mg, 0.2 mmol) in MeOH (2.56 mL). The solution was stirred at room temp. for 24–27 h until no starting material was detectable by TLC and was then concentrated to give a violet solid. FC (CH_2Cl_2/MeOH 19:1) afforded 73 mg (67%) of **17** as a slightly pink solid. TLC (AcOEt): R_f 0.10. MS (ESI$^+$): *m*/*z* (%) = 1163 (10), 582 (100, [M + H]$^+$), 360 (12). HR-MS (ESI$^+$, [M + H]$^+$): 582.2223 ($C_{33}H_{32}N_3O_7$ requires: 582.2240).

Fluoren-9-ylmethyl 5-[Bis(4-methoxyphenyl)(phenyl)methoxymethyl]-4-hydroxy-2-{2-[2-(4-nitrophenyl)ethoxy]pyridin-5-yl}pyrrolidine-1-carboxylate (18): DMT-Cl (35 mg, 0.1 mmol) was added in three portions (16.3 mg) every 30 min to a solution of **17** (50 mg, 0.1 mmol) in dry pyridine (0.5 mL). Additional portions were added every hour until no starting material was detectable by TLC. The solution was diluted with AcOEt (5 mL) and washed with water (10 mL), and the aqueous phase was extracted with AcOEt (5 mL). The combined organic phases were dried with $MgSO_4$ and concentrated to give a yellow oil. The product was subjected to FC (AcOEt/THF 1:0 to 4:1) to yield 56 mg (74%) of **18** as a white foam. The FC column was conditioned with elution solvent + 1% Et_3N. TLC (AcOEt/THF 4:1): R_f 0.38. MS (ESI$^+$): *m*/*z* (%) = 906 (40, [M + Na]$^+$), 273 (78), 215 (100). HR-MS (ESI$^+$, [M + Na]$^+$): 906.3337 ($C_{54}H_{49}N_3O_9Na$ requires: 906.3366).

Fluoren-9-ylmethyl 5-[Bis(4-methoxyphenyl)(phenyl)methoxymethyl]-4-[(2-cyanoethoxy)(diisopropylamino)phosphanyloxy]-2-{2-[2-(4-nitrophenyl)ethoxy]pyridin-5-yl}pyrrolidine-1-carboxylate (19): *i*Pr_2NEt (55 μL, 0.3 mmol) was added to a solution of **18** (56 mg, 0.06 mmol) in dry THF (1.4 mL), followed by CEP-Cl (35 μL, 0.2 mmol). After 2 h, a solution of aq. $NaHCO_3$ (1 M, 5 mL) was added and the product was extracted with AcOEt (2 × 5 mL). The org. phases were dried with $MgSO_4$ and the solvents were evaporated. The resulting yellow foam was purified by FC (AcOEt/hexane 9:1) to afford 56 mg (82%) of **19** as a white foam. The FC column was conditioned with elution solvent + 1% Et_3N. TLC (AcOEt/hexane 9:1): R_f 0.46, 0.52. ^{31}P NMR (161.9 MHz, CDCl3): δ = 147.84, 147.90, 148 ppm.

2-(2-Aminopyridin-5-yl)-4-(*tert*-butyldimethylsilanyloxy)-5-(*tert*-butyldimethylsilanyloxymethyl)pyrrolidine (20): *n*BuLi (1.6 M in hexane, 8.51 mL, 13.6 mmol) was added dropwise at –78 °C to a solution of **12** (4.30 g, 13.6 mmol) in dry THF (60 mL). After 1 h, a solution of **4** (1.56 g, 4.5 mmol) in dry THF (20 mL) was added slowly to the first solution and the mixture was stirred at –78 °C for 2 h. The reaction was quenched with 80 mL of water, the temperature was allowed to reach room temperature, and the mixture was stirred for an additional hour. The layers were then separated and the aqueous layer was extracted with ether (80 mL). The combined org. phases were dried with $MgSO_4$ and concentrated to give a yellow oil. FC, first with AcOEt/hexane 1:4, allowing 1.19 g of remaining **4** (76%) to be recovered, and then with AcOEt/THF 1:0 to 4:1, gave 378 mg (19%) of the desired product **20**. TLC (AcOEt): R_f 0.19. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.05, 0.06, 0.07, 0.07 (4 × s, 12 H, 4 × CH_3-Si), 0.89, 0.90 (2 × s, 18 H, 2 × $(CH_3)_3$-C), 1.71–1.81 (m, 1 H, 3-Hβ), 1.93–2.01 (m, 2 H, 3-Hα, NH), 3.10–3.15 (m, 1 H, 5-H), 3.57–3.69 (m, 2 H, CH_2O), 4.21–4.25 (m, 1 H, 4-H), 4.30–4.37 (m, 3 H, 2-H, NH_2), 6.47 (d, $J_{H,H}$ = 8.5 Hz, 1 H, 3′-H), 7.46 (dd, $J_{H,H}$ = 2.3, 8.5 Hz, 1 H, 4′-H), 8.02 (d, $J_{H,H}$ = 2.3 Hz, 1 H, 6′-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = –5.43, –4.73, –4.60 (4 × CH_3-Si), 18.01, 18.28 (2 × $(CH_3)_3C$-Si), 25.82, 25.90 (2 × $(CH_3)_3$C-Si), 43.68 (C-3), 57.82 (C-2), 64.47 (CH_2O), 68.85 (C-5), 74.26 (C-4), 108.55 (C-3′), 129.39 (C-5′), 136.52 (C-4′), 146.26 (C-6′), 157.58 (C-2′) ppm. Difference-NOE (400 MHz, $CDCl_3$, 25 °C): δ = 3.10–3.15 (5-H) → 3.57–3.69 (4.8%, CH_2O), 4.21–4.25 (2.8%, 4-H), 4.30–4.37 (2.6%, 2-H); 3.57–3.69 (CH_2O) →3.10–3.15 (4.9%, 5-H), 4.21–4.25 (3.4%, 4-H); 6.47 (3′-H) → 7.46 (9.6%, 4′-H); 7.46 (4′-H) → 1.71–1.81 (1.6%, 3-Hβ), 4.30–4.37 (1.5%, 2-H), 6.47 (10.4%, 3′-H); 8.02 (6′-H) → 1.71–1.81 (0.9%, 3-Hβ), 4.30–4.37 (4.3%, 2-H). MS (ESI$^+$): *m*/*z* (%) = 438 (100) [M + H]$^+$, 324 (26), 255 (13), 192 (20). HR-MS (ESI$^+$, [M + H]$^+$): 438.2986 ($C_{22}H_{44}N_3O_2Si_2$ requires: 438.2972).

Fluoren-9-ylmethyl 2-(2-Aminopyridin-5-yl)-4-(*tert*-butyldimethylsilanyloxy)-5-(*tert*-butyldimethylsilanyloxymethyl)pyrrolidine-1-carboxylate (21): A solution of Fmoc-OSu (583 mg, 1.7 mmol) in THF (9 mL) was added to a suspension of **20** (378 mg, 0.9 mmol) in dioxane (9 mL) and aq. $NaHCO_3$ (1 M , 9 mL). After 30 min, sat. aq. NaCl (75 mL) was added and the product was extracted with 3 × 40 mL AcOEt. The org. phase was dried ($MgSO_4$) and the solvents were evaporated. The obtained yellow foam was purified by FC (AcOEt/hexane 6:4) to yield 425 mg (75%) of **21** as a white foam. TLC (AcOEt): R_f 0.55. MS (ESI$^+$): *m*/*z* (%) = 1321 (15), 660 (100) [M + H]$^+$, 433 (30). HR-MS (ESI$^+$, [M + H]$^+$): 660.3662 ($C_{37}H_{54}N_3O_4Si_2$ requires: 660.3652).

Fluoren-9-ylmethyl 4-(*tert*-Butyldimethylsilanyloxy)-5-(*tert*-butyldimethylsilanyloxymethyl)-2-[2-(2-phenoxyacetylamino)pyridin-5-yl]-pyrrolidine-1-carboxylate (22): Pac_2O (664 mg, 2.3 mmol) was added to a solution of **21** (425 mg, 0.6 mmol) in dry pyridine (6.4 mL). After 2 h, the reaction was quenched with water (10 mL) and the mixture was concentrated. The residue was dissolved in AcOEt (120 mL), washed with aq. NaOH (0.1 M , 2 × 16 mL), dried ($MgSO_4$) and concentrated to yield a yellow oil. FC (AcOEt/hexane 1:4) afforded 506 mg (99%) of **22** as a white foam. TLC (AcOEt/hexane 3:7): R_f 0.53. MS (ESI$^+$): *m*/*z* (%) = 794 (55) [M + H]$^+$, 739 (91), 381 (100), 319 (16). HR-MS (ESI$^+$, [M + H]$^+$): 794.4027 ($C_{45}H_{60}N_3O_6Si_2$ requires: 794.4020).

Fluoren-9-ylmethyl 4-Hydroxy-5-(hydroxymethyl)-2-[2-(2-phenoxyacetylamino)pyridin-5-yl]pyrrolidine-1-carboxylate (23): Aq. HCl (1 M, 220 μL) was added to a solution of **22** (126 mg, 0.2 mmol) in MeOH (2.2 mL) and the solution was stirred at room temp. As soon as all the starting material had reacted, according to TLC (20 to 24 h), the pink solution was concentrated and the resulting pink foam was purified by FC (CH_2Cl_2/MeOH 19:1 to 9:1) to yield 89 mg (99%) of **23** as a slightly yellow foam. TLC (AcOEt/THF 9:1): R_f 0.28. MS (ESI$^+$): *m*/*z* (%) = 566 (100) [M + H]$^+$, 149 (34), 121 (34). HR-MS (ESI$^+$, [M + H]$^+$): 566.2294 ($C_{33}H_{32}N_3O_6$ requires: 566.2291).

Fluoren-9-ylmethyl 5-[Bis(4-methoxyphenyl)(phenyl)methoxymethyl]-4-hydroxy-2-[2-(2-phenoxyacetylamino)pyridin-5-yl]pyrrolidine-1-carboxylate (24): DMT-Cl (199 mg, 0.6 mmol) was added portionwise over 1 h to a solution of **23** (277 mg, 0.5 mmol) in dry pyridine (2.7 mL). After an additional hour, the solution was diluted with AcOEt (20 mL) and washed with water (45 mL), and the aqueous phase was extracted with AcOEt (20 mL). The combined organic layers were treated with brine (30 mL) and dried with $MgSO_4$, and the solvents were evaporated. FC (AcOEt/hexane 7:3 to 8:2) of the yellow foam afforded 372 mg (88%) of **24** as a white foam. The FC column was conditioned with elution solvent + 1%

Et_3N. TLC (AcOEt): R_f 0.62. MS (ESI^+): *m/z* (%) = 868 (7) [M + H]$^+$, 609 (100). HR-MS (ESI^+, [M + H]$^+$): 868.3557 ($C_{54}H_{50}N_3O_8$ requires: 868.3597).

Fluoren-9-ylmethyl 5-[Bis(4-methoxyphenyl)(phenyl)methoxymethyl]-4-[(2-cyanoethoxy)(diisopropylamino)phosphanyloxy]-2-[2-(2-phenoxyacetylamino)pyridin-5-yl]pyrrolidine-1-carboxylate (25): *i*Pr_2NEt (373 μL, 2.1 mmol) was added to a solution of **24** (372 mg, 0.4 mmol) in dry THF (9.5 mL), followed by CEP-Cl (239 μL, 1.1 mmol). After 2 h, a solution of aq. $NaHCO_3$ (1 M, 30 mL) was added and the product was extracted with AcOEt (2 × 30 mL). The organic phases were dried with $MgSO_4$ and concentrated to give a colourless oil. FC (AcOEt/hexane 1:1) afforded 412 mg (90%) of **25** as a white foam. The FC column was conditioned with elution solvent + 1% Et_3N. TLC (AcOEt/hexane 1:1): R_f 0.33, 0.42. ^{31}P NMR (161.9 MHz, $CDCl_3$): δ = 147.95, 148.08, 148.30 ppm. MS (ESI^+): *m/z* (%) = 1068 (100) [M + H]$^+$, 359 (41), 341 (56), 313 (33), 253 (32), 200 (25). HR-MS (ESI^+, [M + H]$^+$): 1068.4706 ($C_{63}H_{67}N_5O_9P$ requires: 1068.4676).

Fluoren-9-ylmethyl 2-(2-Aminopyridin-5-yl)-4-hydroxy-5-(hydroxymethyl)pyrrolidine-1-carboxylate (26): Aq. HCl (1 M, 900 μL) was added to a solution of **22** (511 mg, 0.6 mmol) in MeOH (9 mL) and the solution was stirred at room temp. After 30 h, the pink solution was concentrated and the resulting pink foam was purified by FC (CH_2Cl_2/MeOH 19:1 to 9:1) to yield 277 mg (76%) of **23** (described earlier), together with 64 mg (23%) of **26** as a white solid. TLC (CH_2Cl_2/MeOH 19:1): R_f 0.04. MS (ESI^+): *m/z* (%) = 432 (100) [M + H]$^+$, 254 (21), 210 (10).

2-(2-Aminopyridin-5-yl)-4-hydroxy-5-(hydroxymethyl)pyrrolidine (27): Piperidine (94 μL, 1.0 mmol) was added to a suspension of **26** (61 mg, 0.1 mmol) in DMF (650 μL) and the mixture was stirred at room temp. overnight. The solution was then concentrated to yield a brown solid. FC (AcOEt/MeOH 1:0 to 1:1) afforded 21 mg (71%) of **27** as a slightly yellow solid. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.04. ^1H NMR (300 MHz, MeOD, 25 °C): δ = 2.17–2.25 (m, 1 H, 3-Hα), 2.39–2.50 (m, 1 H, 3-Hβ), 3.55–3.60 (m, 1 H, 5-H), 3.85 (d, $J_{H,H}$ = 4.9 Hz, 2 H, CH_2O), 4.42–4.45 (m, 1 H, 4-H), 4.76 (dd, $J_{H,H}$ = 6.0, 12.1 Hz, 1 H, 2-H), 6.69 (d, $J_{H,H}$ = 8.8 Hz, 1 H, 3′-H), 7.68 (dd, $J_{H,H}$ = 2.4, 8.8 Hz, 1 H, 4′-H), 8.03 (d, $J_{H,H}$ = 2.3 Hz, 1 H, 6′-H) ppm. ^{13}C NMR (75 MHz, MeOD, 25 °C): δ = 41.24 (C-3), 61.01 (C-2), 61.59 (CH_2O), 70.82 (C-5), 73.80 (C-4), 110.71 (C-3′), 121.23 (C-5′), 139.09 (C-4′), 148.15 (C-6′), 161.66 (C-2′) ppm. Difference-NOE (400 MHz, MeOD, 25 °C): δ = 2.17–2.25 (3-Hα) → 2.39–2.50 (15.6%, 3-Hβ), 4.42–4.45 (2.2%, 4-H), 4.76 (6.5%, 2-H); 2.39–2.50 (3-Hβ) → 2.17–2.25 (19.5%, 3-Hα), 4.42–4.45 (9.3%, 4-H), 4.76 (2.1%, 2-H), 7.68 (6.0%, 4′-H), 8.03 (3.2%, 6′-H); 3.55–3.60 (5-H) → 3.85 (4.0%, CH_2O), 4.42–4.45 (2.6%, 4-H), 4.76 (4.0%, 2-H); 3.85 (CH_2O) → 3.55–3.60 (4.8%, 5-H), 4.42–4.45 (3.0%, 4-H); 4.42–4.45 (4-H) → 2.17–2.25 (1.4%, 3-Hα), 2.39–2.50 (4.8%, 3-Hβ), 3.55–3.60 (2.5%, 5-H), 3.85 (2.8%, CH_2O); 4.76 (2-H) → 2.17–2.25 (4.7%, 3-Hα), 3.55–3.60 (3.5%, 5-H), 7.68 (2.6%, 4′-H), 8.03 (5.8%, 6′-H); 6.69 (3′-H) → 7.68 (7.6%, 4′-H); 7.68 (4′-H) → 2.39–2.50 (2.9%, 3-Hβ), 4.76 (3.4%, 2-H), 6.69 (9.8%, 3′-H); 8.03 (6′-H) → 2.39–2.50 (2.1%, 3-Hβ), 4.76 (7.0%, 2-H). MS (ESI^+): *m/z* (%) = 210 (100) [[M + H]$^+$], 192 (88), 122 (21). HR-MS (ESI^+, [M + H]$^+$): 210.1246 ($C_{10}H_{16}N_3O_2$ requires: 210.1242).

Oligonucleotides: All oligodeoxyribonucleotides were prepared from the modified phosphoramidites **19** or **25** and commercially available natural building blocks on (deoxy)nucleoside-CPG solid supports (Glen Research). Synthesis was performed in the trityl-off mode on the 1.3 μmol scale of a Pharmacia LKB Gene Assembler Special DNA-synthesiser or on the 1.0 μmol scale of a PerSeptive Biosystems Expedite Nucleic Acid Synthesis System by standard solid-phase phosphoramidite chemistry. Modifications to the synthesis cycle were introduced for the incorporation of the nonnatural building blocks as follows: (i) the coupling time was extended from 1.5 to 6 min, (ii) (*S*-benzylthio)-1*H*-tetrazole (0.25 M in CH_3CN) was used as activator, and (iii) for the sequences containing dp2AP, the classical acetyl capping step was replaced by a phenoxyacetyl (Pac) capping step (Capping A solution: 6.15 g DMAP in 100 mL CH_3CN; Capping B solution: 7.5 g phenoxyacetic anhydride and 10 mL *sym*-collidine in 40 mL CH_3CN). Standard ammonia deprotection (33% aq. ammonia at 55 °C for 16 h) led to cleavage of the crude oligonucleotides from the solid support and removal of all protecting groups (including Fmoc). For all sequences containing NPE protective groups, a two-step deprotection procedure first involving treatment with TDB (1 M) in acetonitrile at 60 °C for 8 h, followed by ammonolysis, was applied. The crude oligonucleotides were purified by ion exchange HPLC (DEAE-HPLC) (ET 125/4 NUCLEOGEN DEAE 60–7 column, 125 × 4.0 mm, Macherey–Nagel) or FPLC (Mono Q HR 5/5 column, Pharmacia biotech) and were then desalted over Sep-Pack C18 cartridges (Waters) according to the manufacturer's protocol. Purified oligonucleotides were monitored for purity by reversed-phase HPLC (Aquapore RP-300, 7 μm, 220 × 4.6 mm, Brownlee or NUCLEOSIL 100–5, C18, 220 × 5 mm, Macherey–Nagel) or FPLC (Pep RPC HR 5/5 column, Pharmacia biotech). The integrity of all oligonucleotides was confirmed by ESI-MS.

Thermal Denaturation Experiments: UV-melting experiments were carried out with a Varian Cary 100 BIO UV/Vis spectrophotometer equipped with a temperature controller. Data were collected with the Cary WinUV thermal software. All measurements were conducted in buffered solution (140 mM KCl, 7 mM NaH_2PO_4, 0.5 mM $MgCl_2$) at oligonucleotide concentrations of 1.2 μM; the pH values were measured directly in the sample. Melting curves were recorded at 260 nm in a consecutive heating-cooling-heating cycle (0–90 °C or 20–90 °C) with a temperature gradient of 0.5 °C min^{-1}. For temperatures <20 °C, nitrogen was passed through the spectrophotometer to avoid condensation. To avoid evaporation of the solutions, 6–8 drops of dimethylpolysiloxane were added at the top of the samples in the cell. The absorbance melting curves were smoothed and the first derivative curves were obtained by use of Varian WinUV or Microcal Origin software.

Circular Dichroism Spectroscopy: CD spectra were recorded with a Jasco J-715 spectropolarimeter equipped with a Jasco PFO-350S temperature controller. The temperature was measured directly in the sample. The graphs were subsequently smoothed with a noise filter. The buffer was used as blank.

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[1] D. Praseuth, A. L. Guieysse, C. Helene, *Biochim. Biophys. Acta* **1999**, *1489*, 181–206.
[2] C. Helene, N. T. Thuong, A. Harel-Bellan, *Ann. N. Y. Acad. Sci.* **1992**, *660*, 27–36.
[3] J. M. Kalish, M. M. Seidman, D. L. Weeks, P. M. Glazer, *Nucleic Acid Res.* **2005**, *33*, 3492–3502.
[4] M. M. Seidman, N. Puri, A. Majumdar, B. Cuenoud, P. S. Miller, R. Alam, *Ann. N. Y. Acad. Sci.* **2005**, *1058*, 119–127.
[5] F. A. Rogers, J. A. Lloyd, P. M. Glazer, *Curr. Med. Chem. Anti-Cancer Agents* **2005**, *5*, 319–326.
[6] J. R. Goñi, X. de la Cruz, M. Orozco, *Nucleic Acid Res.* **2004**, *32*, 354–360.

[7] S. Buchini, C. J. Leumann, *Curr. Opin. Chem. Biol.* **2003**, *7*, 717–726.
[8] K. R. Fox, *Curr. Med. Chem.* **2000**, *7*, 17–37.
[9] J. Robles, A. Grandas, E. Pedroso, F. J. Luque, R. Eritja, M. Orozco, *Curr. Org. Chem.* **2002**, *6*, 1333–1368.
[10] S. Hildbrand, C. J. Leumann, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1968–1970.
[11] S. Hildbrand, A. Blaser, S. P. Parel, C. J. Leumann, *J. Am. Chem. Soc.* **1997**, *119*, 5499–5511.
[12] P. J. Bates, C. A. Laughton, T. C. Jenkins, D. C. Capaldi, P. D. Roselt, C. B. Reese, S. Neidle, *Nucleic Acids Res.* **1996**, *24*, 4176–4184.
[13] S. A. Cassidy, P. Slickers, J. O. Trent, D. C. Capaldi, P. D. Roselt, C. B. Reese, S. Neidle, *Nucleic Acids Res.* **1997**, *25*, 4891–4898.
[14] U. V. Krosigk, S. A. Benner, *J. Am. Chem. Soc.* **1995**, *117*, 5361–5362.
[15] A. Ono, P. O. P. Ts'O, L.-S. Kan, *J. Am. Chem. Soc.* **1991**, *113*, 4032–4033.
[16] A. Ono, P. O. P. Ts'O, L.-S. Kan, *J. Org. Chem.* **1992**, *57*, 3225–3230.
[17] G. Xiang, W. Soussou, L. W. McLaughlin, *J. Am. Chem. Soc.* **1994**, *116*, 11155–11156.
[18] G. Xiang, L. W. McLaughlin, *Tetrahedron* **1998**, *54*, 375–392.
[19] S. Buchini, C. J. Leumann, *Angew. Chem. Int. Ed.* **2004**, *43*, 3925–3928.
[20] R. T. Ranasinghe, D. A. Rusling, V. E. C. Powers, K. R. Fox, T. Brown, *Chem. Commun.* **2005**, 2555–2557.
[21] D. Guianvarc′h, J.-L. Fourrey, R. Maurisse, J.-S. Sun, R. Benhida, *Bioorg. Med. Chem.* **2003**, *11*, 2751–2759.
[22] Y. Wang, D. A. Rusling, V. E. C. Powers, O. Lack, S. D. Osborne, K. R. Fox, T. Brown, *Biochemistry* **2005**, *44*, 5884–5892.
[23] A. B. Eldrup, O. Dahl, P. E. Nielsen, *J. Am. Chem. Soc.* **1997**, *119*, 11116–11117.
[24] A. G. Olsen, O. Dahl, P. E. Nielsen, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1551–1554.
[25] B. Cuenoud, F. Casset, D. Hüsken, F. Natt, R. M. Wolf, K.-H. Altmann, P. Martin, H. E. Moser, *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1288–1291.
[26] M. J. J. Blommers, F. Natt, W. Jahnke, B. Cuenoud, *Biochemistry* **1998**, *37*, 17714–17725.
[27] A. Mayer, A. Häberli, C. J. Leumann, *Org. Biomol. Chem.* **2005**, *3*, 1653–1658.
[28] A. Häberli, C. J. Leumann, *Org. Lett.* **2002**, *4*, 3275–3278.
[29] Q. Wu, C. Simons, *Synthesis* **2004**, *10*, 1533–1553.
[30] V. L. Schramm, P. C. Tyler, *Curr. Top. Med. Chem.* **2003**, *3*, 525–540.
[31] A. Häberli, C. J. Leumann, *Org. Lett.* **2001**, *3*, 489–492.
[32] A. Momotake, H. Togo, M. Yokoyama, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1193–1200.
[33] M. Yokoyama, T. Ikenogami, H. Togo, *J. Chem. Soc. Perkin Trans. 1* **2000**, 2067–2071.
[34] S. Hanessian, R. Margarita, A. Hall, S. Johnstone, M. Tremblay, L. Parlanti, *J. Am. Chem. Soc.* **2002**, *124*, 13342–13343.
[35] S. Hanessian, M. Tremblay, J. F. W. Petersen, *J. Am. Chem. Soc.* **2004**, *126*, 6064–6071.
[36] S. Hanessian, S. Claridge, S. Johnstone, *J. Org. Chem.* **2002**, *67*, 4261–4274.
[37] I. Collado, J. Ezquerra, C. Pedegral, *J. Org. Chem.* **1995**, *60*, 5011–5015.
[38] W. N. Speckamp, M. J. Moolenaar, *Tetrahedron* **2000**, *56*, 3817–3856.
[39] B. A. Horenstein, R. F. Zabinski, V. L. Schramm, *Tetrahedron Lett.* **1993**, *34*, 7213–7216.
[40] R. H. Furneaux, G. Limberg, P. C. Tyler, V. L. Schramm, *Tetrahedron* **1997**, *53*, 2915–2930.
[41] G. B. Evans, R. H. Furneaux, G. J. Gainsford, V. L. Schramm, P. C. Tyler, *Tetrahedron* **2000**, *56*, 3053–3062.
[42] G. B. Evans, R. H. Furneaux, T. L. Hutchison, H. S. Kezar, P. E. J. Morris, V. L. Schramm, P. C. Tyler, *J. Org. Chem.* **2001**, *66*, 5723–5730.
[43] S. Bevers, S. B. Ha, L. W. McLaughlin, *Biochemistry* **1999**, *38*, 7710–7718.
[44] L. L. Kiessling, L. C. Griffin, P. B. Dervan, *Biochemistry* **1992**, *31*, 2829–2834.

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