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Mapping the Landscape of Potentially Primordial Informational Oligomers: (3′→2′)-D-Phosphoglyceric Acid Linked Acyclic Oligonucleotides Tagged with 2,4-Disubstituted 5-Aminopyrimidines as Recognition Elements

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In memory of Leslie Orgel, a quintessential chemical evolutionist

Abstract: The (3′→2′)-phosphodiester glyceric acid backbone containing an acyclic oligomer tagged with 2,4-disubstituted pyrimidines as alternative recognition elements have been synthesized. Strong cross-pairing of a 2,4-dioxo-5-aminopyrimidine hexamer, rivaling locked nucleic acid (LNA) and peptide nucleic acid (PNA), with complementary adenine-containing DNA and RNA sequences was observed. The corresponding 2,4-diamino- and 2-amino-4-oxo-5-aminopyrimidine-tagged oligomers were synthesized, but difficulties in deprotection, purification, and isolation thwarted further investigations. The acyclic phosphate backbone structure of the protected oligomer seems to be prone to an eliminative degradation owing to the acidic hydrogen at the 2′-position—an arrangement that renders the oligomer vulnerable to the conditions used for the removal of the protecting groups on the heterocyclic recognition element. However, the free oligomers seem to be stable under the conditions investigated.

Keywords: aminopyrimidines · base pairing · nucleic acids · oligonucleotides · RNA

Introduction

The postulate of an RNA world, wherein RNA plays both the role of an informational carrier as well as the role of a catalyst has come to be widely accepted.[1] In contrast, the emergence of RNA from potentially natural pathways is still open to debate,[2] which has led to the hypothesis that simpler, transient informational oligomers that are more compatible with potentially prebiological pathways could have prepared the trail for the appearance of RNA.[3] In this context, investigations have been conducted by surveying the base-pairing behavior of a broad spectrum of such "primitive" oligomers.[4a–d] Herein, we describe the synthesis of, and the difficulties encountered en route to, an informational oligomer derived from a (3′→2′)-phosphodiester glyceric acid backbone containing 2,4-disubstituted 5-aminopyrimidines as base-pairing components (Scheme 1).[5] We also document the base-pairing properties of a 2,4-dioxo-5-aminopyrimidine-tagged (3′→2′)-phosphoglycerate oligomer.

This potentially primordial informational oligomer is derived conceptually from 2′- or 3′-phosphoglyceric acid and 2,4-disubstituted 5-aminopyrimdines building blocks (Scheme 2); the formation of these individual starting components within the constraints of prebiotic chemistry has been demonstrated by the groups of Orgel[6] and Miller.[7]

Our inclination to investigate the (3′→2′)-phosphoglycerate-derived oligomer was further strengthened by a qualitative conformational analysis,[8] which revealed that it could adopt potential pairing conformations (Scheme 1). The existence of such acyclic informational oligomeric systems had been anticipated previously (Scheme 3), as a consequence of studies on the lyxopyranosyl nucleic acid[9] and TNA[10] oligonucleotides. One such system, $X = CH_2$ (GNA), the formation of which we had considered not to be compatible with prebiological pathways, has been synthesized and stud-

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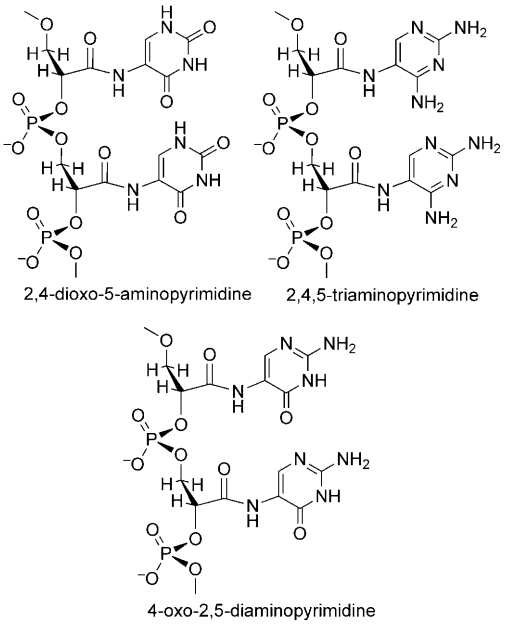
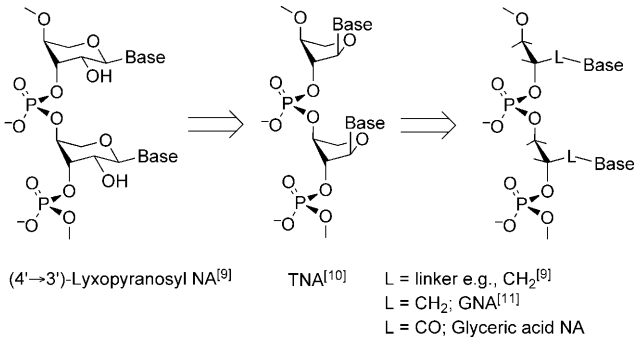
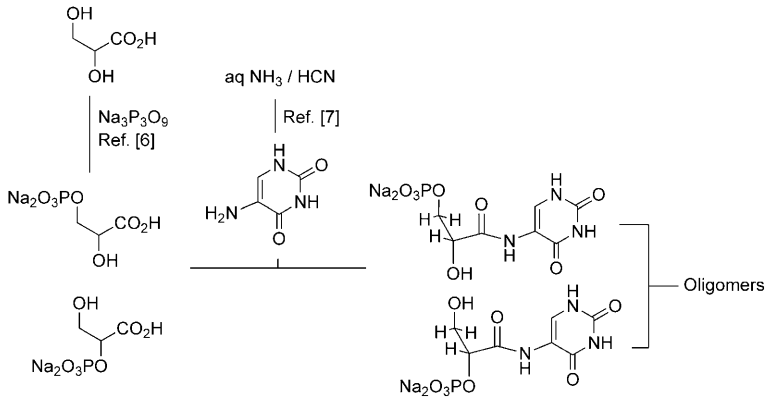


Scheme 1. Idealized conformational representations of the three 2,4-disubstituted-5-aminopyrimidine-tagged 3′→2′ phosphoglycerate backbones under consideration in this investigation.



Scheme 3. Structural simplification of (3′→2′)-threofuranosyl nucleic acid (TNA), which in turn was inspired by studies on (4′→3′)-lyxopyranosyl nucleic acid, leads to a diversity of acyclic informational oligomeric systems. GNA = glycerol nucleic acid, NA = nucleic acid.



Scheme 2. Conceptual derivation of the 2,4-disubstituted 5-aminopyrimidine tagged (3′→2′)-phosphoglycerate backbone from the starting materials shown and through the reaction pathways considered to be geochemically plausible.

ied by Meggers et al.[11] Investigating the base-pairing properties of a constitutionally and generationally simple glyceric acid derived oligomer would be relevant in the context presented above. With this perspective, we began the synthesis and study of the base-pairing properties of these phosphoglycerate oligonucleotides (Scheme 1) by utilizing contemporary and conventional methodologies.

Abstract in Chinese:

本文报道了一系列新型非环寡聚体的合成. 该系列寡聚体以3'→2'磷酸二酯甘油酸为骨架并采用2,4-二取代-5-氨基嘧啶作为识别单元.

含有2,4-二氧-5-氨基嘧啶识别单元的六聚体可与含有腺嘌呤的DNA及RNA序列产生强烈交叉配对. 相应的含有2,4-二氨基-5-氨基嘧啶或2-氨基-4-氧-5-氨基嘧啶的寡聚体也得以合成，而在去保护和纯化与分离中遇到的困难阻碍了进一步研究.

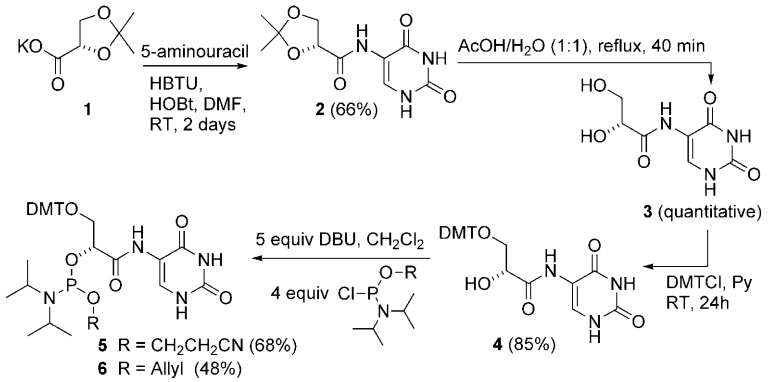
由于2'位氢的酸性，杂环识别单元的选择性去保护反应困难，而寡聚体骨架结构容易同时发生消去性降解.

Results and Discussion

Synthesis of Phosphoramidite Monomers

2,4-Dioxo-5-aminopyrimidine Series

The synthesis of the 2,4-dioxo-5-aminopyrimidine (5-aminouracil)-tagged glycerate derivative **2** started from readily available dimethyl acetonide **1** derived from potassium glycerate (Scheme 4).[12] The coupling of 5-aminouracil with **1** to form the amide was investigated at length, and the use of HBTU with HOBt as coupling and activating agents in DMF as the solvent was found to afford the best results (66% yield of **2**). The deprotection step (heating at reflux in aqueous acetic acid) afforded diol **3** in quantitative yield. Tritylation of diol **3** under standard conditions (pyridine with DMTCl (2 equiv)) was found to give **4** in up to 85% yield. Considerable effort was expended on the last step to produce the cyanoethoxy phosphitylated derivative **5**; excess DBU (5 equiv) and phosphitylating reagent (4 equiv) were found to be necessary to push the reaction to completion. A 0.1 M solution of **5** in acetonitrile was found to be stable for 24 hours at RT (as monitored by TLC). Because the deprotection and isolation of the oligomer prepared with derivative **5** were found to be problematic (see below), we also synthesized the allyloxy derivative **6** (Scheme 4). Both **5** and **6** were utilized to prepare the oligomers in this series.



Scheme 4. Preparation of the phosphitylated building blocks in the 2,4-dioxo-5-aminopyrimidine series. HBTU = *O*-(benzotriazol-1-yl)tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxybenzotriazole, DMF = *N*,*N*-dimethylformamide, DMT = 4,4′-dimethyoxytrityl, Py = pyridine, DBU = 1,5-diazabicyclo-[5.4.0]undec-5-ene.

2,4,5-Triaminopyrimidine Series

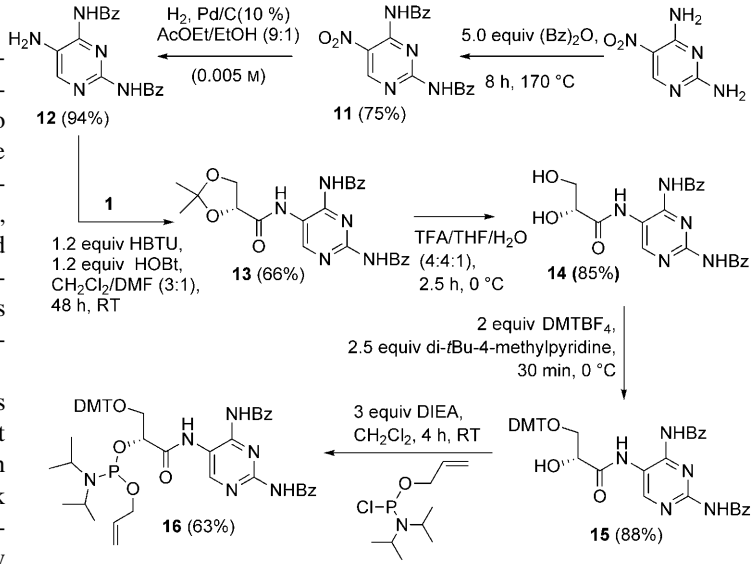
The synthesis of the corresponding 2,4,5-triaminopyrimidine[13]-containing monomer building block was not straightforward (Scheme 5). For example, whereas we were able to successfully couple the 2,4,5-triaminopyrimidine to prepare diol **9**, further tritylation of this diol under a variety of conditions gave none of the expected 3′-tritylated derivative, but surprisingly the 2′-tritylated compound **10** (as confirmed by ^{1}H–^{1}H COSY; see the Supporting Information, Figure S87). The influence of the heterocycle (2,4-dioxo- versus 2,4-diaminopyrimidine), and the complete reversal of the regioselectivity of the tritylation are remarkable.

Phosphitylation of **10** under a variety of conditions was unsuccessful with the generation of complex mixtures that indicated interference from the 2,4-diamino moieties, which necessitated the use of protecting groups. This is in stark contrast to the behavior of this heterocycle in the oligodipeptide series, in which no protecting groups were necessary on the monomeric building blocks to prepare the requisite oligomers.[4d]

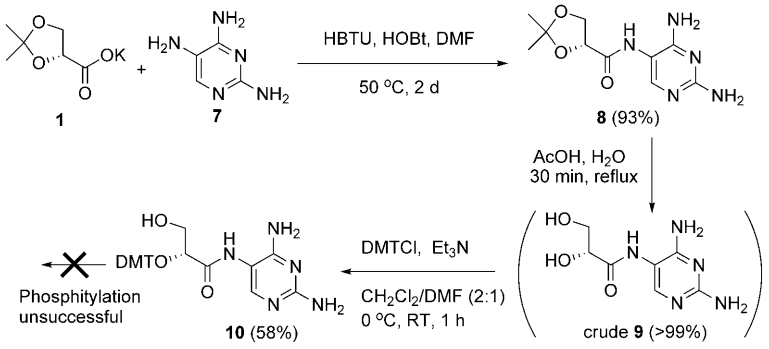
We explored various protecting groups (such as benzoyl, allyloxylcarbonyl, and formamidinyl) and settled on the N^2,N^4-dibenzoyl derivative **13** as a suitable a target (Scheme 6). Direct benzoylation of **8**, after many trials and variations, afforded not the expected 2,4-N^2,N^4-dibenzoyl derivative **13**, but a mixture of N^4-bisbenzoylated and N^4-benozylated products, which could not be converted to **13** under a spectrum of reaction conditions. Therefore, we decided to synthesize N^2,N^4-dibenzoyl-5-aminopyrimidine (**12**), which then could be coupled to **1**. The preparation of **12** started from the commercially available 2,4-diamino-5-nitropyrimidine.

Whereas the benzoylation proceeded uneventfully, the reduction step (of the nitro group) provided a small twist to afford a mixture of the desired product along with the N^2,N^4,N^5-tribenzoylated and N^4-benzoylated products. Under the usual (0.1 M) concentrations, an intermolecular transfer from the N^2-benzoyl group to the newly produced, more reactive 5-amino group was found to take place.[13] Such an unwanted reaction was easily suppressed by running the reaction under dilute (0.005 M) concentrations to afford 94% of **12**.

The synthesis proceeded as planned (Scheme 6), but not without operational troubles.

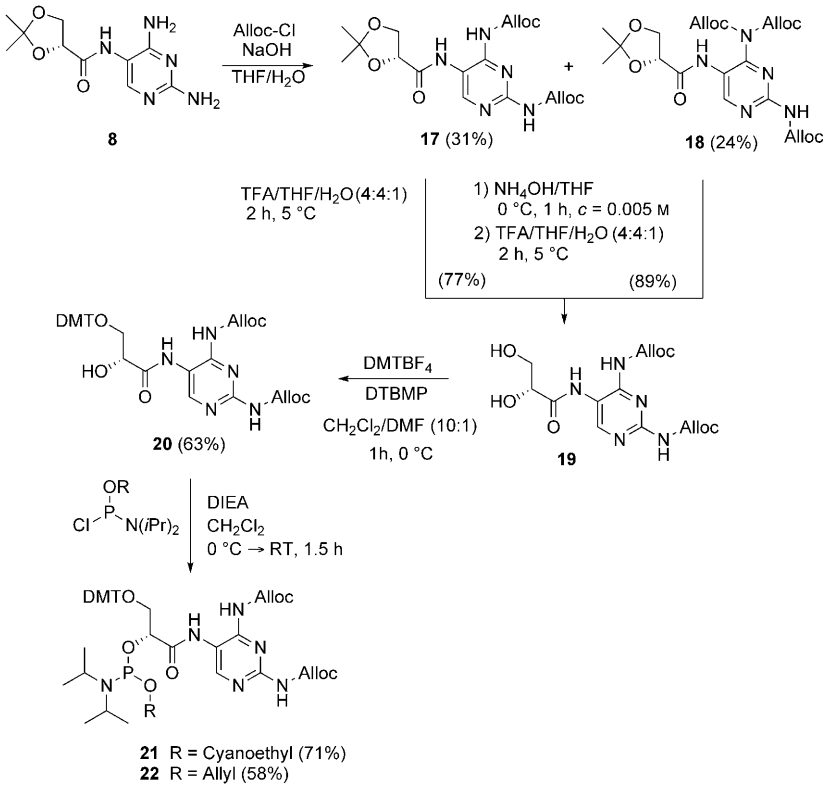


Scheme 6. Synthesis of the N^2,N^4-dibenzoylated phosphoramidite derivative **16**. Bz = benzoyl, THF = tetrahydrofuran, TFA = trifluoroacetic acid, DIEA = ethyldiisopropylamine.



Scheme 5. Unsuccessful attempts at preparing the phosphoramidite derivative in the 2,4,5-triaminopyrimidine series emphasize the need to protect the 2,4-diamino moieties.

The tritylation step was rendered difficult by the reluctance of diol **14** to be tritylated under a variety of conditions, which indicated interference from the heterocyclic ring. It was overcome by the use of $DMTBF_4$ and a hindered base (di-*tert*-butyl-4-methylpyridine (DTBMP))[14] to get the desired regiochemistry of **15** in 88% yield this was then converted into phosphoramidite **16**. The phosphitylation step was fraught with difficulties with respect to purification and isolation. However, the desired compound **16** was isolated by minimizing the contact time with silica gel and cooling the chromatography column. The use of **16** in the oligomer synthesis revealed an unforeseen problem of the stability of the oligomer under the conditions for the removal of the benzoyl protecting group (see below).



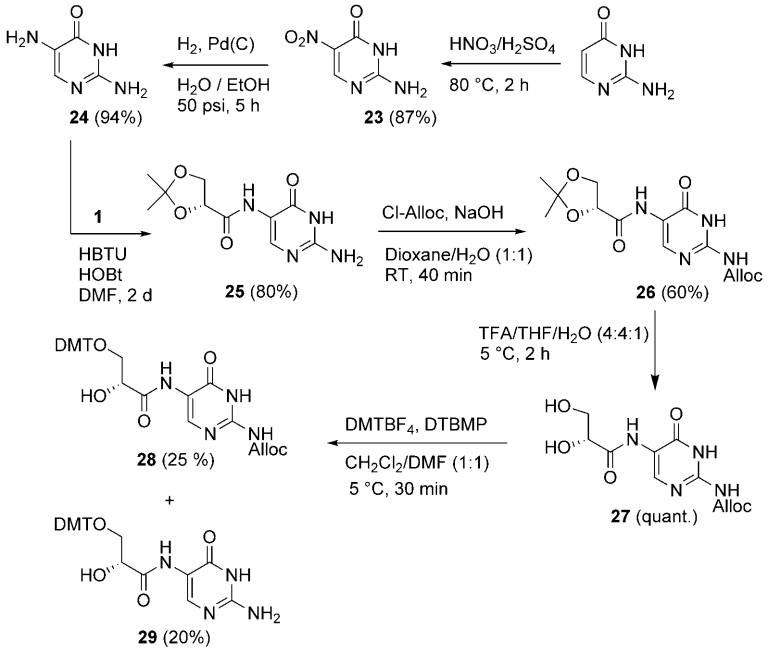
Scheme 7. Synthesis of the allyloxycarbonyl derivatives **21** and **22**. Alloc = allyloxycarbonyl.

To overcome this drawback, we turned to the allyloxycarbonyl protecting groups that are known to be compatible with the automated solid-support synthesis of RNA/DNA, and are removed under mild deprotection protocols.[15] Derivative **8** was subjected to the allyloxycarbonylation reaction. Initially this reaction gave all possible mixtures of products (see the Supporting Information, page 65). After some fine-tuning we could, under biphasic reaction conditions, obtain the diallyloxycarbonyl derivative **17** and triallyloxycarbonyl derivative **18** in about 55% overall yield (Scheme 7). Whereas ketal **17** could be converted directly into the desired diallyloxycarbonyl-protected diol **19**, the triallyloxycarbonyl ketal **18** was first treated with aqueous NH_3 to remove one of the two allyloxycarbonyl groups at the N^4-position and was then converted into diol **19**. The ketal deprotection reactions had to be conducted at low temperatures for short reaction times; longer reaction times (4 h) or reactions at RT led to the loss of one allyloxycarbonyl protecting group along with some hydrolysis of the amide bond. Subsequent tritylation of the primary alcohol of **19** followed by phosphitylation of **20** afforded monomers **21** and **22**, which were used in the preparation of oligomers.

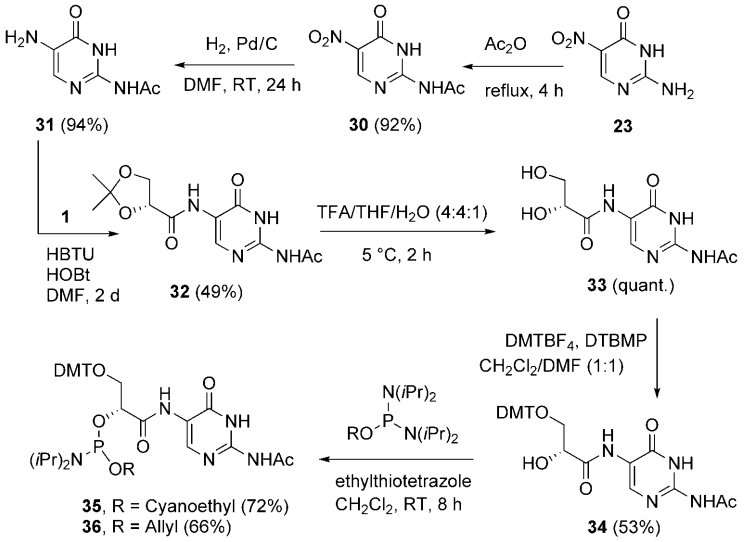
4-Oxo-2,5-diaminopyrimidine Series

We then turned our attention to 4-oxo-2,5-diaminopyrimidine, a recognition element that is known to exhibit self-pairing.[4d] The synthesis started with isocytosine, which was nitrated with sulfonitric mixed acid, followed by reduction of the nitro group (Scheme 8). Coupling of **24**[4d,16] with the protected potassium glycerate salt **1**, led to amide **25**. This compound was protected with allyloxycarbonyl in a mixture of dioxane/water as the solvent in which product **26** precipitates (all attempts to perform the protection under anhydrous conditions with common bases such as DIEA or DBU were unsuccessful). The ketal was deprotected under acidic conditions to afford diol **27**. Tritylation of **27** with $DMTBF_4$ proceeded with high conversion (one spot on the TLC plate), but isolation of product **28** was not quantitative. Purification by column chromatography led to only 25% yield, along with a side product (**29**), which had lost the allyloxycarbonyl protecting group. Moreover, conducting the purification with 2% Et_3N afforded only the deprotected compound **29**. Phosphitylation of **28** was unsuccessful under a variety of conditions.

The problems encountered above were attributed to the lability of the allyloxycarbonyl group in **28**, which led us to search for a more stable protecting group for the 2-amino moiety. We chose the acetate derivative because the synthesis of the acetate derivative **31** has been reported by heating nitroisocytosine **23** at reflux in acetic anhydride followed by reduction of the nitro group (Scheme 9).[17] Coupling with the glyceric acid salt afforded ketal **32**, which was deprotected under acidic conditions to afford diol **33**. Tritylation of diol **33** posed technical problems because of solubility issues, but was resolved by using a 1:1 mixture of CH_2Cl_2 and DMF. For the last step, the use of standard chlorophosphoramidite reagent failed presumably due to interference from the 4-oxo group of the heterocycle (several signals in



Scheme 8. Synthesis of the phosporamidites in the 4-oxo-2,5-diaminopyrimidine series with 2-*N*-allyloxylcarbonyl protecting group.



Scheme 9. Synthesis of phosphoramidites in the 4-oxo-2,5-diaminopyrimidine series with 2-*N*-acetyl protecting group.

the ^{31}P NMR spectrum). This problem was overcome by changing the phosphitylation reagent to the less reactive bis(diisopropylamine)-functionalized phosphoramidite,[18] and by using the acid activator ethylthiotetrazole, which afforded the desired monomers **35** and **36** (Scheme 9) with which were used in oligomer synthesis.

Synthesis and Isolation of Oligomers

With phosphoramidite monomers **5**, **6**, **16**, **21**, **22**, **35**, and **36** in hand, we began exploring the machine-assisted solid-support synthesis of the hexamers with appropriate modifications to the protocols supplied by the manufacturer (see the Supporting Information). We used both the universal solid support[19] and the commercially available solid support loaded with appropriate DNA nucleosides.

2,4-Dioxo-5-aminopyrimidine ($^{5\text{-aP}}$OO) Series gly-($^{5\text{-aP}}$OO)$_n$

We began exploring the synthesis of a dodecamer gly-($^{5\text{-aP}}$OO)-12mer using phosphoramidite **5**. However, the coupling efficiency was not optimal (approx. 80% per step). HPLC monitoring of the deprotection and detachment from solid support by NH_3 (H_2O/EtOH, 3:1) at 4°C indicated that there was a substantial amount of short oligomers and none of the desired longer oligomers. The MALDI-TOF spectrum confirmed this and revealed an interesting pattern of the parent signals being accompanied by a corresponding [*M*+54] signal, which was attributed to the presence of a CH_2CH_2CN moiety (see the Supporting Information, Figure S91). This pointed to an eliminative fragmentation of the oligomer (at the phosphotriester stage) occurring before the deprotective removal of the cyanoethoxy group. Changing the deprotecting conditions did not overcome this limitation.

Therefore, we attempted to use the allyloxy group, which could be selectively cleaved first by the use of palladium chemistry,[15] resulting in a phosphodiester-linked oligomer that would be more stable to the conditions of detachment from the solid support. Using phosphoramidite **6**, we were able to synthesize the gly-($^{5\text{-aP}}$OO)-6mer with an average coupling yield of about 85%. Deprotection, detachment, purification by HPLC, and desalting afforded sufficient

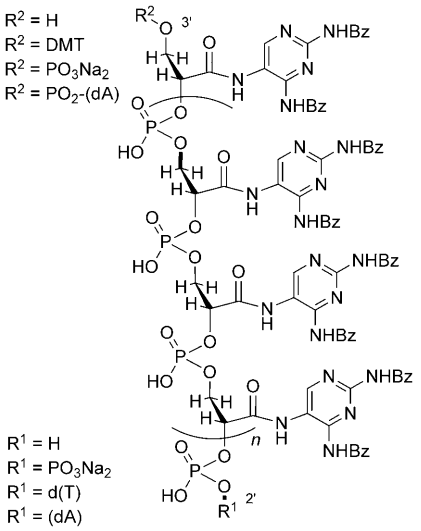
amounts of the hexamer (see the Supporting Information, Figure S93) for base-pairing studies to be conducted.

2,4,5-Triaminopyrimidine ($^{5\text{-}aP}$NN) Series gly-($^{5\text{-}aP}$NN)$_n$

The solid-support synthesis of a hexamer starting from **16** proceeded well (an average coupling yield of 89%, as monitored by trityl assay). After the palladium-catalyzed removal of the allyl protecting groups on the phosphate backbone, we faced problems with the deprotection/detachment step (aq NH_3/EtOH, 3:1 at RT) to produce the fully deprotected hexamer (Scheme 10, $R^1=R^2=H$), as evidenced by HPLC traces and the mass spectrum of the crude reaction mixture (see the Supporting Information, Figures S94 and S95), although these condition had been shown to be compatible with 5-aminouracil-tagged glycerate backbones. Various deprotection conditions ($MeONH_2$, $MeNH_2$, NH_2NH_2 at 0°C or RT) for the removal of benzoyl groups at the 2,4-diamino positions always resulted in extensive decomposition of the hexamer (see the Supporting Information, Figure S96).

To prevent the possibility that the end 3′-OH group of the hexamer was acting as an internal nucleophile and destroying the hexamer, we also prepared hexamers with four different end groups on solid support (Scheme 10): 1) the end 3′-OH group was protected as O-DMT, 2) the end 3′-OH group was phosphorylated, 3) the end 3′-OH group was coupled to a deoxyadenosine monomer, and 4) the 3′- and 2′-OH groups were protected with deoxyadenosine phosphate. We also prepared a hexamer for which the synthesis started with a deoxythymidine-containing CPG (controlled-pore glass) support with the final 3′-OH group protected as a phosphate.

The syntheses of these hexamers proceeded with 90–95% average coupling efficiency. However, the deprotection conditions for the removal of the benzoyl groups always led to



Scheme 10. End group variations in the 2,4,5-triaminopyrimidine series in an attempt to prevent the decomposition of the oligomers during removal of the protecting groups and detachment from solid support. d(T) = deoxythymidine, (dA) = deoxyadenosine.

the decomposition of the oligomer; this indicated that the protection of the end OH groups did not help in preventing the destruction of the hexamer. MALDI-TOF data indicated incomplete removal of the N^2,N^4-benzoyl groups. We propose (based on earlier experiences with 2,6-diaminopurine)[20] that the benzoyl group at the N^2-position is removed easily, which makes the pyrimidine ring more electron rich (when compared with 2-NHBz); the N^4-benzoyl group acquires a strong "amide bond" like character. Thus, the benzoyl group at the N^4-position becomes difficult to remove under the usually mild conditions used in oligonucleotide chemistry. This idea was tested by the reaction of the DMT derivative of the N^2,N^4-dibenzoyl-protected monomer **15** by subjecting it to various benzoyl deprotection conditions. After removal of the first NHBz group, harsh conditions (NH_2NH_2 or $MeNH_2$ at 60°C) were necessary for the removal of the second NHBz group; these conditions were hostile to the stability of the acyclic phosphodiester backbones.

To circumvent this problem, we used derivative **22** and began with the synthesis of an gly-($^{5\text{-}aP}$NN)-6mer with the hope that the milder conditions for the removal of allyl group, which had been successful in the 2,4-dioxo-5-aminopyrimidine series, would also work in the 2,4,5-triaminopyrimidine series. The automated oligomer synthesis (using a universal solid support) proceeded with greater than 90% average coupling efficiency (trityl assay). Nevertheless, after the palladium-catalyzed allyl deprotection procedures and detachment from solid support with aqueous NH_3 in EtOH very little oligomer was obtained (see the HPLC traces in the Supporting Information, Figure S96). The use of alternative procedures to remove the allyl protecting group also failed to yield any improvement in the yield of the oligomers (as monitored by HPLC and MALDI-TOF spectrometry).

We then tried a different approach to obtain the gly-($^{5\text{-}aP}$NN)-6mer starting with derivative **21**. The hexamer was capped with d(T) at the terminus (to prevent any decomposition initiated by a free OH group at the end position). Once again the oligomer synthesis proceeded without any problems (>90% average coupling efficiency). However, after the allyl deprotection and detachment protocols (aqueous solutions of NH_4OH, $MeNH_2$, or N_2H_4 with EtOH (3:1) or Et_3N/H_2O/EtOH, 1.5:1.5:1, RT), the results were the same as with the previous ($^{5\text{-}aP}$NN)-6mer starting with monomer **21**; no regular pattern or sharp signals were observed in HPLC traces (see the Supporting Information, Figure S97). A mass spectrum of the crude product displayed signals corresponding to fragments of oligomers of lower molecular weight (without the allyloxycarbonyl or allyl protecting groups).

We sought to understand the difficulty in the deprotection process; to that end, a sample of solid support (1 mg; without the palladium treatment for removing the allyloxycarbonyl groups) was suspended in aqueous NH_3 at RT for 2.5 h. The HPLC analysis with 1/3 of the solution showed extremely small amounts of UV-active material present and

the mass analysis did not show signals corresponding to the desired hexamer. Another set of experiments was carried out to compare results between 1) the full palladium-catalyzed deprotection protocol; 2) the full palladium-catalyzed deprotection protocol, but without the sodium diethyldithiocarbamate washing step; and 3) full deprotection protocol, but with a different catalyst, $[Pd(PPh_3)_4]$, for the removal of the allyloxycarbonyl groups. In all three cases the same signal patterns were observed in the HPLC traces with very little of the signal (UV-active material) that would correspond to the retention time of the desired hexamer. We also attempted a SN_2' reaction with mercaptoethanol because of a literature precedent for the removal of the allyl protecting group at the phosphate;[21] but this approach was not successful in our hands.

We checked the efficiency of the palladium-catalyzed deprotection of the monomeric compound **17** and found that the removal of the allyloxycarbonyl group under identical conditions proceeded smoothly to afford **8**. This observation suggested that the problems we were facing were at the oligomeric level and indicated the instability of the acyclic phosphate triester backbone under these conditions. Compound **17** was also subjected to treatment with aqueous NH_4OH, $MeNH_2$, or N_2H_4 to check for the removal of the allyloxycarbonyl groups by nucleophilic substitution (as carried out with the benzoyl groups previously). However, these experiments gave a mixture of compounds that could only be accounted for as urea-type derivatives formed at the amino groups at C_2, C_4, or both; the best result was achieved by using hydrazine to give 50% yield of the desired deprotected product **8** and 50% of a mono-semicarbazide derivative.

We have not been able to prepare an phosphoglyceric acid oligomer tagged with 2,4,5-triaminopyrimidine. This failure is attributed mainly to the instability of the oligomer under the various conditions employed for the removal protecting groups on the 2,4,5-triaminopyrimidine heterocycle. This instability may be a function of the 2,4,5-triaminopyrimidine heterocycle because the corresponding 2,4-dioxo-5-aminopyrimidine-tagged oligomers have been shown to be isolable under comparable deprotection conditions.

4-Oxo-2,5-diaminopyrimidine ($^{5\text{-}aP}NO$) Series gly-($^{5\text{-}aP}NO$)$_n$

Oligomer synthesis was first attempted by using the cyanoethyl derivative **35** (Scheme 9). We intended to study the modes of deprotection and stability of the oligomer under different conditions. We synthesized a gly-($^{5\text{-}aP}$NO)-4mer with the DMT in place (to avoid strand scission that may be caused by the terminal OH). The oligomer synthesis proceeded with greater than 90% average efficiency per step. The solid-support-containing oligomer was deprotected (aq NH_3/EtOH, 3:1) at room temperature; HPLC traces after 22 hours showed no further change. MALDI-TOF analysis showed not only the signal for the desired oligomer without the final DMT group (under mass measurement conditions the DMT group falls off), but also other signals corresponding to partial removal of the acetate and cyanoethyl groups. Various deprotection conditions were tested (NH_2NH_2 at 5°C or $MeNH_2$ (44% aq)/EtOH (3:1) at RT). $MeNH_2$ treatment showed a sharp signal at 19 minutes in the HPLC trace, and correspondingly the expected mass for the unprotected 4-mer in the mass spectrum (see the Supporting Information, Figure S98); thus, this treatment became the method of choice. We then attempted the synthesis of a gly-($^{5\text{-}aP}NO)_6$-mer capped with d(T) starting with phosphoramidite **35**. The trityl assay showed 91% average efficiency per coupling step. We employed the methylamine deprotection protocol to obtain the free oligomer. HPLC analysis showed that after 4 hours at RT, 3 sharp signals appeared at 21, 22 and 23 minutes (see the Supporting Information, Figure S99d); after purification and desalting, these were assigned to the NO-4mer (gly-($^{5\text{-}aP}NO)_4$-d(T)), NO-5mer (gly-($^{5\text{-}aP}NO)_5$-d(T)), and the desired NO-6mer (gly-($^{5\text{-}aP}NO)_6$-d(T)).

To improve on these results and minimize the fragmentation of the oligomer, we explored the following conditions: 1) an aqueous solution of NH_3 at longer reaction times, 2) pretreatment with a solution of Et_3N in anhydrous CH_3CN, 3) an aqueous solution of Et_3N, and 4) *tert*-butylamine (see the Supporting Information, Table S5). The best results were obtained with Et_3N in anhydrous acetonitrile, which removes selectively the cyanoethyl group to afford the phosphodiester-linked oligomer, followed by exposure to methylamine at RT for 4 hours (see the Supporting Information, Figure S100). The crude oligomer was purified by ion-exchange HPLC and desalted over Sephadex by size-exclusion chromatography. The MALDI-TOF mass of this oligomer could only be acquired if the sodium ions were completely exchanged by treatment with ion-exchange resin (IR-120) in the triethylammonium form, as documented in Figure 1.

Base-Pairing Studies

The base-pairing properties of these oligomers (see the Supporting Information, Table S6) were investigated by the use of temperature-dependent UV and circular dichroism (CD) absorption spectroscopy in 10 mM Na_2HPO_4 (pH 7.0) buffer containing 1 M NaCl and 0.1 mM Na_2EDTA (EDTA = ethylenediaminetetraacetic acid).

2,4-Dioxo-5-aminopyrimidine-Tagged Hexamer Series 3′-gly-($^{5\text{-}aP}OO)_6$-2′-OPO_3^{2-}

We compared the temperature-dependent UV absorption behavior of the hexamer alone and in the presence of the corresponding complementary DNA and RNA sequences and determined that 260 nm was the appropriate wavelength that should be used to measure the melting temperature, T_m (Table 1 and Figure 2). Base pairing with both complementary adenine-containing poly-(dA) and poly-(rA) sequences was observed, as evidenced by the sigmoidal UV melting curves. However, we were surprised by the magnitude of the strength of cross-pairing, as indicated by the UV-T_m melting values, which translated to about 10°C per base pair with

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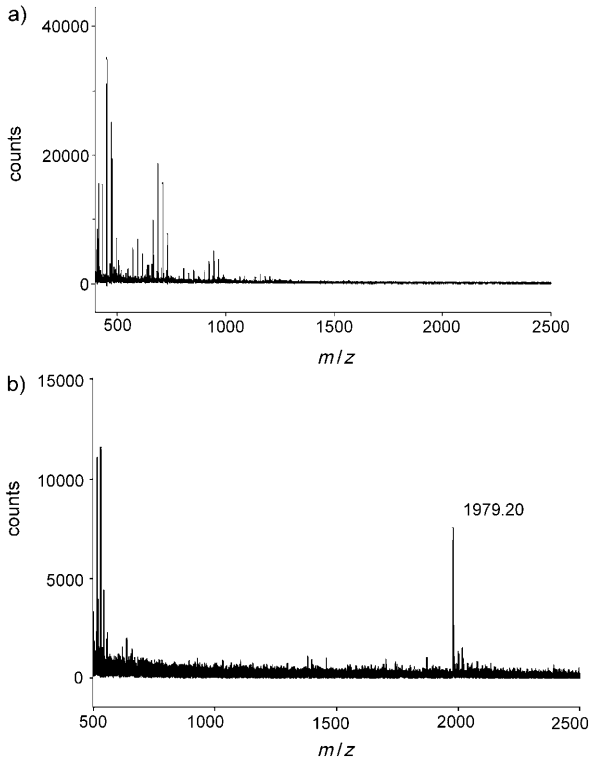


Figure 1. MALDI-TOF spectra of gly-($^{5\text{-aP}}$NO)$_6$-d(T) a) before and b) after treatment with IR-120-(Et_3NH^+) resin.

Table 1. Inter-system duplex formation by (3′→2′)-phosphoglycerate-tagged 2,4-dioxo-5-aminopyrimidine hexamer (3′-gly-($^{5\text{-aP}}$OO)$_6$-2′-OPO_3^{2-}) with DNA and RNA as documented by melting temperature values and their comparison with the cross-pairings of peptide nucleic acid (PNA) and locked nucleic acid (LNA).

	Duplex[a]	UV-T_m [°C][b]	CD-T_m [°C][b]
1	3′-gly-($^{5\text{-aP}}$OO)$_6$-2′-OPO_3^{2-}+poly-(dA)	63.6 (55.3)	58
2	3′-gly-($^{5\text{-aP}}$OO)$_7$-2′-OPO_3^{2-}+poly-(dA)	69.4 (58.9)	–
3	3′-gly-($^{5\text{-aP}}$OO)$_6$-2′-OPO_3^{2-}+dA$_{12}$	52.7[c] (15.7)	52
4	3′-gly-($^{5\text{-aP}}$OO)$_6$-2′-OPO_3^{2-}+poly-(rA)	44.4	45
5	3′-gly-($^{5\text{-aP}}$OO)$_6$-2′-OPO_3^{2-}+TNA–D$_{12}$	24.0[c]	21
6	DNA–T$_{16}$+dA$_{16}$	55.3[10b]	
7	DNA–T$_{16}$+rA$_{16}$	59.3[10b]	
8	(PNA) H-T$_6$-Lys-NH$_2$+dA$_6$	31.0[d,22]	
9	(LNA) T$^L_5$T+dA$_{14}$	32.0[e,23]	
10	(LNA) T$^L_5$T+dA$_{14}$	40.0[e,23]	

[a] D = 2,6-diaminopurine, A = adenine, T = thymine, T^L = LNA–thymidine. [b] Measured in 10 mM Na_2HPO_4 (pH 7.0) buffer containing 1 M NaCl and 0.1 mM Na_2EDTA; total oligonucleotide concentration is 10 μM (1:1 ratio), unless indicated otherwise. Melting temperatures were obtained from maxima of the first derivative of the curve (Kaleidagraph software). UV-T_m Values in brackets are from cooling curve. CD spectra obtained at 5 °C temperature intervals. [c] 5 μM+2.5 μM. [d] 0.18 OD_{260nm} each,10 mM $MgCl_2$, 140 mM NaCl, pH 7.4. [e] 100 mM NaCl, 0.1 mM EDTA (total oligonucleotide concentration is 3 μM, 1:1 ratio).

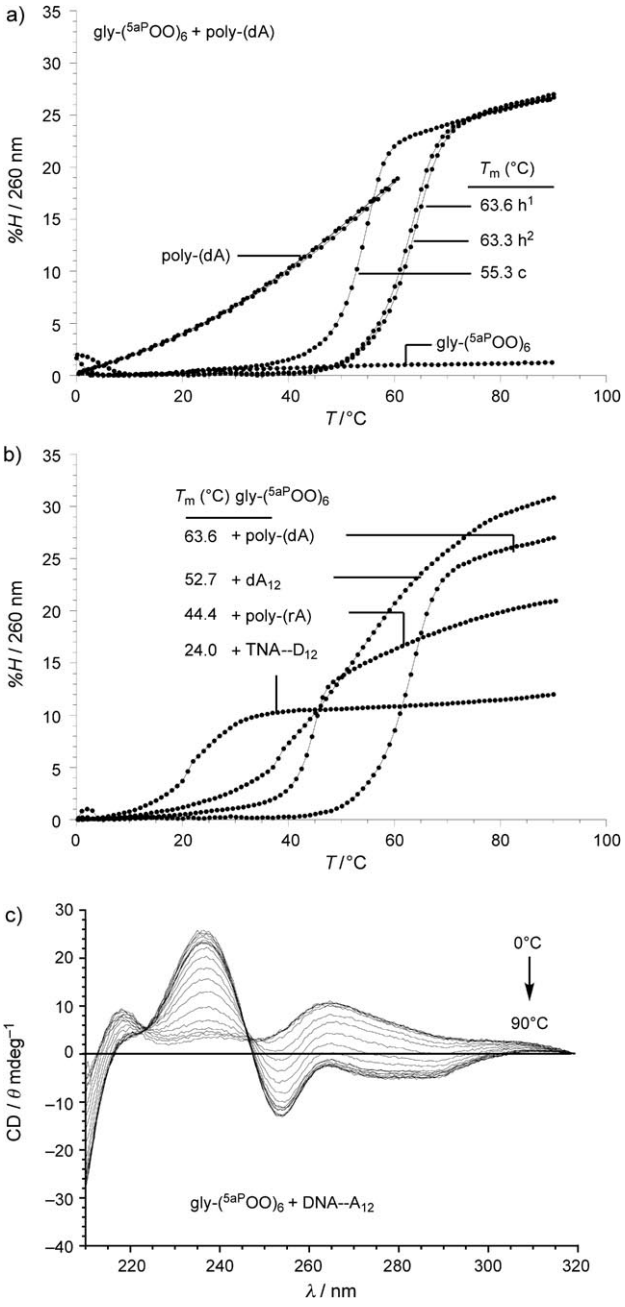


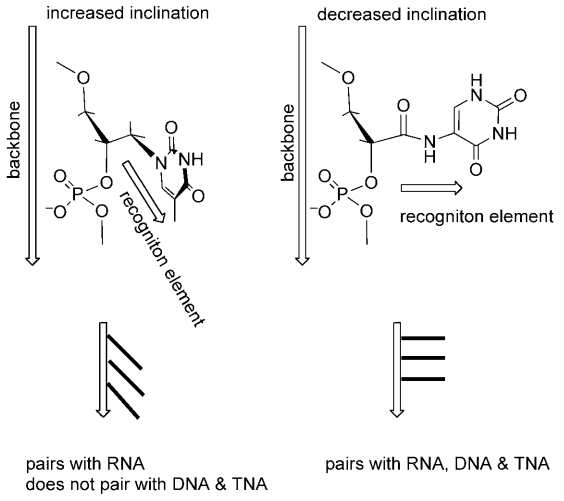
Figure 2. Base-pairing behavior of the gly-($^{5\text{-aP}}$OO)$_6$ oligomer. a) UV spectroscopic T_m curves documenting a strong interaction with poly-(dA), exhibiting hysteresis between heating (h^1 and h^2) and cooling (c) curves; b) UV-T_m (heating) curves demonstrating different pairing affinity towards RNA, DNA, and TNA; c) Temperature-dependent CD spectroscopy of the duplex formed with dA$_{12}$. Measurements conditions: see Table 1. No self-pairing was observed for individual partner strands.

poly-(dA) and about 7 °C per base pair with poly-(rA). Such strong base-pairing behavior seems to rival those exhibited by PNA[22] and LNA,[23] and far exceeding the corresponding DNA–DNA and RNA–DNA interactions.

UV-T_m values also indicated that there was a preference exhibited by this phosphoglycerate backbone for DNA (more flexible backbone) over RNA (less flexible backbone). In agreement with this trend of weaker base-pairing interaction with conformationally less flexible systems, the hexamer was found to pair even weaker with dodecamer of TNA–diaminopurine (TNA–D$_{12}$), which was considered to

be even less flexible than RNA.[10] Not surprisingly, the hysteresis that was exhibited in the UV-T_m curves in the cross-pairings with DNA was absent in the measurements with RNA and TNA as partners (see the Supporting Information, Figure S101). Correspondingly, the temperature-dependent CD spectra of complexes formed by 3′-gly-($^{5\text{-aP}}$OO)$_6$-2′-OPO_3^{2-} with poly-(dA), poly-(rA), and TNA–D_{12} indicated that their overall shapes were different from each other (see the Supporting Information, Figure S102).

It is noteworthy that this acyclic phosphoglycerate backbone, which resembles GNA, behaves very differently in its cross-pairing capabilities towards RNA, DNA, and TNA when compared with GNA. Whereas GNA cross-pairs only with RNA[11] and not DNA[11] or even TNA,[24] the (3′→2′)-phosphoglycerate-tagged 2,4-dioxo-5-aminopyrimidine backbone base pairs with all three systems, with DNA being the strongest partner. Such a drastic difference in behavior of these two acyclic backbones can be attributed to the alteration of the backbone/recognition-element axes (Scheme 11).



Scheme 11. Comparison of the idealized conformations of the GNA and (3′→2′)-phosphoglycerate backbones reveals differences in the inclination of the backbone/base-pair axes between the two systems, which may be responsible for their contrasting cross-pairing behavior.

There is less-pronounced inclination of the backbone/base-pair axes in the phosphoglycerate oligomer than GNA,[11] which seems to restore the cross-pairing abilities with DNA and TNA. This would also be consistent with the observation that DNA (which has less backbone/base-pair axes inclination than RNA[25]) pairs more strongly with the phosphoglycerate oligomer than RNA or TNA.

A Job plot of dA_{12} with gly-($^{5\text{-aP}}$OO)$_6$, clearly showed the formation of a triplex, as expected from the stoichiometry of the partners involved (Figure 3); however, only one melting temperature was observed by UV and CD spectroscopy (Table 1, entry 3).

The variation in the UV-T_m values with variation in length of sequences (Table 1, entries 1–3) and the temperature-dependent CD spectra (see the Supporting Information, Figure S102) give additional evidence of base-pairing interactions between the strands. The 3′-gly-($^{5\text{-aP}}$OO)$_6$-2′-OPO_3^2 hexamer alone gave no sigmoidal behavior, both in UV-T_m (Figure 2a) and CD-T_m measurements (see the Supporting Information, Figure S103), which indicated a lack of self-association. The hexamer was found to be stable under the measurement conditions and had not undergone any decomposition (see the Supporting Information, Figure S104).

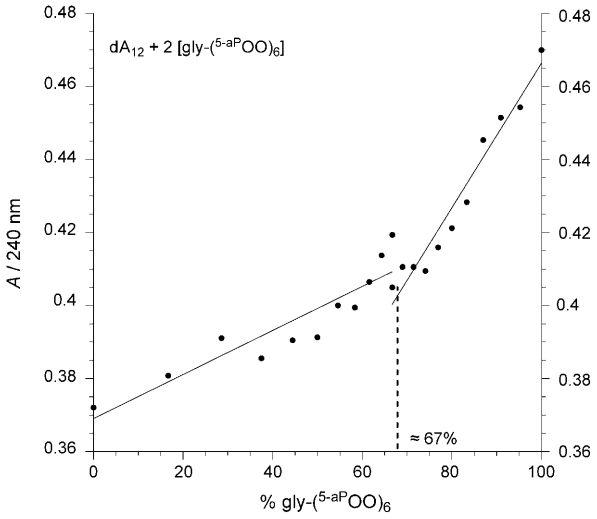


Figure 3. Job plot of dA_{12} and gly-($^{5\text{-aP}}$OO)$_6$ showing the 2:1 ratio of the pairing partners for the intersystem homotriplex formed at 2 °C (monitored at 240 nm); for measurement conditions, see Table 1.

4-Oxo-4,5-aminopyrimidine Series

With the 5mer gly-($^{5\text{-aP}}$NO)$_5$-dT in hand, we explored the base-pairing properties. UV-T_m measurements were conducted at six different wavelengths: a shallow transition was obtained and the sigmoidal curve patterns could not be reproduced in subsequent experiments. However, the corresponding temperature-dependent CD spectrum suggested a cooperative melting behavior—again no clear CD-T_m value could be discerned.

When the base-pairing studies were conducted with the 4-oxo-2,5-diamino hexamer, gly-($^{5\text{-aP}}$NO)$_6$-dT, the temperature-dependent UV absorption curves were reproducible (repeated 3 times) and showed the same behavior over the 3 different experiments to give a very shallow transition melting temperature at around 44 °C (Figure 4a). Further confirmation was obtained from temperature-dependent CD spectroscopy (a shallow CD-T_m between 40–50 °C; Figure 4b). After the various melting experiments (7 heating–cooling cycles), the purity and stability of gly-($^{5\text{-aP}}$NO)$_6$-dT was checked by HPLC (see the Supporting Information, Figure S105), which showed no decomposition after the repeated heating–cooling–heating cycles.

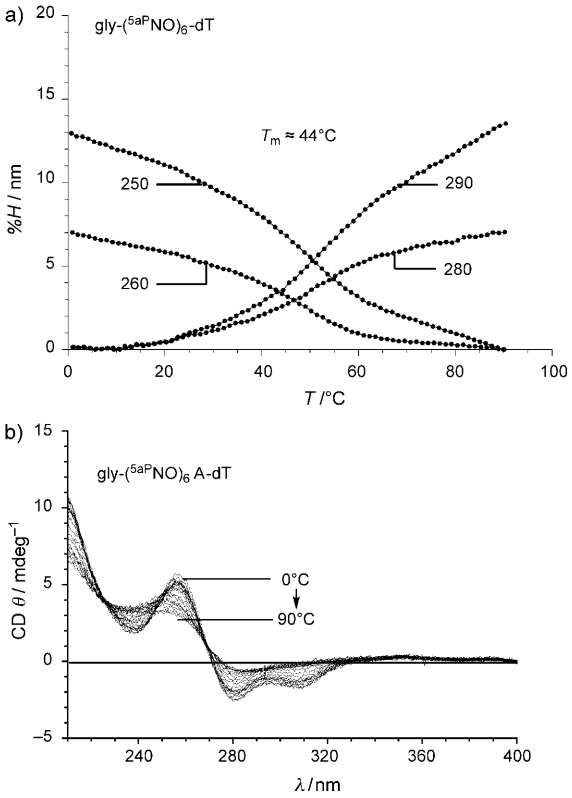


Figure 4. Intrasystem self-pairing behavior of the gly-($^{5\text{-aP}}$NO)$_6$-dT oligomer. a) UV spectroscopic melting (h^2) curves at various wavelengths; no hysteresis was observed. b) Temperature-dependent CD spectroscopy curves. For measurement conditions, see Table 1.

Conclusion

The (3′→2′)-phosphodiester glyceric acid backbone containing an acyclic oligomer tagged with 2,4-disubstituted pyrimidines as alternative recognition elements have been synthesized and the base-pairing behavior was investigated. Strong cross-pairing of the 2,4-dioxo-5-aminopyrimidine hexamer, on a par with LNA and PNA, with adenine-containing DNA and RNA sequences was observed. Self-pairing was found in the 2,5-diamino-4-oxopyrimidine hexamer series. Further investigations were thwarted by system-specific synthetic difficulties, for example, the 2,4-dioxo-5-aminopyrimidine-incorporated oligomer could be accessed by using the allyloxy, but not the cyanoethoxy, protecting group on the phosphoramidite. However, neither this approach nor any variation of the protecting group chemistry was successful in obtaining the 2,4,5-triaminopyrimidine-containing oligomers. The 2,5-diamino-4-oxopyrimidine-tagged oligomers could be synthesized by using the cyanoethyl phosphoramidite derivatives with an acetyl protecting group. From our extended investigations, we conclude that the protection and deprotection chemistry that is needed to access the oligomers in this series have been the stumbling block. The acyclic backbone structure of these protected oligomers seems to be prone to an eliminative degradation of the phosphate backbone owing to the acidic hydrogen at the 2′-position. This propensity seems to lie at the heart of our troubles in accessing enough of these oligomers for an extended study. Nevertheless, the oligomers (once synthesized, isolated, and purified) seem to be stable for further base-pairing studies. Base-pairing results indicate that they do harbor the potential to function as informational oligomeric systems. These observations notwithstanding, the difficulties encountered in the synthetic endeavor to obtain adequate quantities of these oligomers have led us to relinquish further study of these systems.

Acknowledgements

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