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Design, Synthesis, and Hybridisation of Water-Soluble, Peptoid Nucleic Acid Oligomers Tagged with Thymine

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The preparation of a new class of backbone-modified PNA mimetic incorporating thymine is described. Target dipeptoid monomer **21** was synthesised from an *N*-[2-(thymin-1-yl)ethyl]glycinate ester and a properly protected iminodiacetic acid. The distinctive structural motif in the backbone is a carboxy group, inserted to impart water solubility to the oligomer. Two achiral oligopeptoid sequences (8-mer and 12-mer), characterised by the shift of the amide carbonyl group away from the nucleobase, were efficiently assembled according to solid-phase synthesis protocols. Thermal denaturation studies showed that the two homopyrimidine oligopeptoids do not effectively hybridise with complementary sequences of DNA and RNA or fully synthetic (2,4-diamino)-triazin-6-yl-tagged peptoid **22**. A possible reason could reside in the concurrent unfavourable influence of the anionic *N*-(carboxymethyl) moieties and the flexible nucleobase/backbone ethylene linker.

Introduction

Peptide nucleic acid (PNA, **1**; Figure 1) is a synthetic nucleic acid mimic built on a polyamide backbone.[1] The remarkable hybridisation and biostability properties make PNA of considerable interest in several areas of chemistry, biology and medicine.[2] However, despite its many excellent properties, PNA has two serious limitations: low water solubility[3] and poor cellular uptake.[4] A significant number of PNA derivatives have been designed and synthesised to circumvent these drawbacks, leading to a variety of promising analogs.[5] Common strategies include conjugation of PNA termini with polar moieties,[6] elaboration of PNA/DNA chimeras[7] and addition of polar side chains onto the pseudopeptide PNA backbone.[1,2,8] In the last approach, the enantiotopic H atoms of the *N*-(2-aminoethyl)glycine are often replaced by charged groups.[9] When D-lysine[10a] or D-arginine[5b,10b,10c] is introduced, a stabilizing effect is observed in the PNA-DNA hybridisation, both for conformational preferences [a (*P*)-helix is formed][11] and for the presence of positive charges along the backbone.[12] On the other hand, negatively charged PNA derivatives (hypNA-pPNA)[13] are showing promise in antisense experiments in cell culture, demonstrating that anionic backbones do not necessarily deteriorate the hybridisation properties of nucleopseudopeptides.

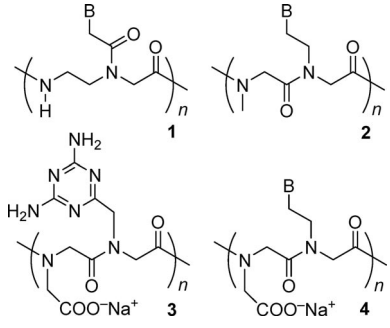

Figure 1. Structures of PNA (**1**), *N*-methyl peptoid nucleic acid (**2**), triazine-tagged *N*-(carboxymethyl) peptoid nucleic acid (**3**) and thymine-based *N*-(carboxymethyl) peptoid nucleic acid (**4**). B = nucleobase.

Since no conclusive data are yet available concerning the relevance of specific molecular features in the PNA structure, and considering the excellent hybridisation properties of neutral and polyanionic, pure-α-peptoid[14] backbones (**2**[15] and **3**,[16] Figure 1), we decided to investigate novel PNA backbones consisting of *N*-alkylated glycine oligomers with alternating N^1-(2-aminoethyl)thymine and *N*-(carboxymethyl) residues (**4**). We opted for a PNA mimic made of an integral peptoid backbone, in order to compare

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the recognition abilities of **4** (once mixed with the proper polyadenylated strand), with the known, fully peptoidic **2**[15] and **3**,[16] both capable of hybridising nucleic acids.

The exchange of the backbone methylene group with the side chain carbonyl group, the elimination of the secondary amide linkage and the anionic nature of the oligomers, constitute risky features for successful hybridisation experiments. The first two traits, in particular, abolish the H-bond donor capacity of the backbone. However, no spectral[17] or crystallographic evidence[18] has ever supported a supposed "strong H bond between the side-chain carbonyl oxygen atom (α to the methylene group carrying the base) and the backbone amide of the next residue" (**5**; Figure 2), as reported by Almarsson and co-workers.[14]

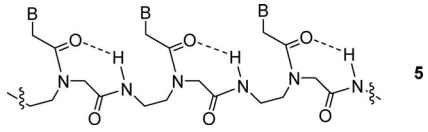

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Figure 2. Hypothesised H bonds between the side-chain carbonyl oxygen atoms and the backbone amides.

Furthermore, two enlightening studies on PNAs showing N^{γ} alkylation,[19] indicated that the N–H H-bonding was not crucial for the stabilization of the PNA/nucleic acid double strand. Thus, we reasoned that *N*-alkylation of the backbone with polar groups, in a pure peptoid framework, could represent a novel strategy for a fine-tuning of PNA properties and provide a new way to overcome the pressing water-solubility issue.

In the design of the proposed peptoid oligomer **4**, we chose the highly hydrophilic *N*-(carboxymethyl) side chain. The excellent pairing properties showed by the (2,4-diamino)triazin-6-yl/*N*-methylencarboxy-tagged peptoid **3**[16] encouraged us to insert anionic residues. The thymine residues were attached to the oligoglycine backbone through an ethyl linker, so that they were separated by the same number of bonds found in nucleic acids (Figure 3, bold black bonds). The spacing between the recognition units on the peptoid framework was the same number of bonds as that present in DNA (Figure 3, bold grey bonds).

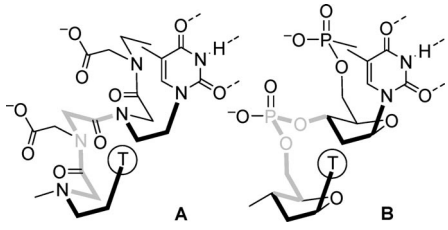
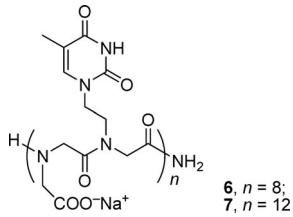


Figure 3. Backbone thymine positioning in the designed peptoid oligomer (**A**) and A-type DNA (**B**).[20]

In the present work, we report the synthesis, the solid-phase oligomerisation and the base-pairing behaviour of two oligomeric peptoids **6** and **7** (Figure 4), carrying eight and twelve repetitive units, respectively. In order to evaluate their pairing properties, hybridisation assays with complementary sequences of DNA, RNA and synthetic (2,4-diamino)triazin-6-yl-tagged oligopeptoid **3** were carried out. Preliminary theoretical studies were performed on models based on the reported, solution-phase NMR structure of the antiparallel DNA/PNA duplex carrying the sequence 5′-d(GACATAGC)-3′/NH_2(CTGTATCG)-COOH.[17b]



6, *n* = 8;
7, *n* = 12

Figure 4. Structures of target oligomers **6** and **7**.

Results and Discussion

Computational Studies

We performed preliminary molecular dynamics (MD) studies in order to evaluate the ability of the thymine-tagged oligoglycine strand to form a stable duplex with polyadenylated DNA strands. We used the solution-phase NMR structure of the 5′-d(GACATAGC)-3′/NH_2(CTGTATCG)-COOH[17b] duplex as a reference model to estimate the performance of calculation parameters (simulation time, solvation model) and correctly interpret the computational outcomes. On the double-stranded octanucleotide under investigation, we performed MD calculations (10 ns, 300 K) in the presence of water [generalized Born/surface area (GB/SA) model].[21] The inspection of the MD trajectory revealed that the duplex was stable throughout the simulation, even though we used no distance restraints in the calculations. We observed, at 10 ns, a little perturbation at the edge of the duplex, consisting in a deviation from the planarity of two base pairs (Figure 5, NMR model). The MD results showed that, in the NMR model, the duplex preserved its arrangement with respect to the set of H bonds between the base pairs. This outcome confirmed the stability of the reference structure and the reliability of our computational methodology.

At this point, we turned our attention to models presenting an oligoglycine backbone and exchanged the side-chain amide carbonyl groups with the methylene groups of the polyamide framework, obtaining a peptoid skeleton. In a first model, we substituted the N^{γ} with a methyl group (Figure 6, model **C**, similar to the Xu oligomer,[15] except for the nucleobases). In a second model, we linked the same nitrogen atom to an *N*-(carboxymethyl) side chain, giving the backbone present in our target oligomers **6** and **7** (Figure 6, model **D**).

The results of the MD calculations (10 ns, 300 K) showed that the two models preserved the gross double-helix arrangement (Figure 5, models **C** and **D**). However, the presence, in the repetitive, six-atom, peptoid framework of four

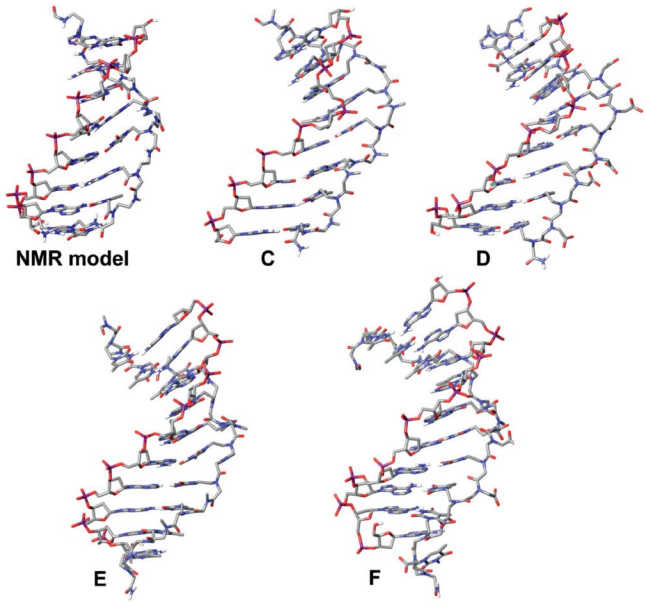


Figure 5. View of hybrid duplex structures for the solution-phase NMR conformation (NMR model), **C**, **D**, **E** and **F** models, after MD calculation (10 ns, 300 K). The models are represented by tubes and coloured by atom type (C, gray; polar H, white; N, dark blue; O, red; P, purple). The nonpolar H atoms have been omitted for clarity.

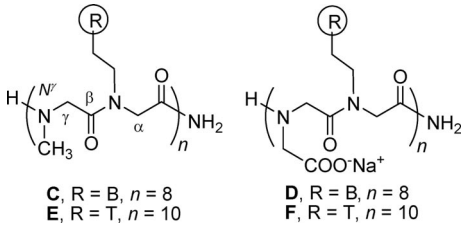


Figure 6. Models used in the MD calculations. For the nucleobase (B) in **C** and **D**, see the solution-phase NMR structure solved by Eriksson and Nielsen.[17b] T = thymine.

sp^2-hybridised atoms, instead of three in the related PNA, the addition of *N*-methyl or *N*-acetate groups and, above all, the fact that there were conformationally demanding ethylene linkers separating the nucleobase from the backbone, induced in models **C** and **D** a slightly larger helical pitch in the peptoid counterpart of the double helix. We note that the anionic *N*-(carboxymethyl) side chain of model **D**, establishing favourable electrostatic interactions with the solvent, positively contributed to duplex stability (when compared with model **C**, see the Supporting Information). Similarly to the DNA phosphates, the carboxylates projected the nucleobases towards the hydrophobic core of the duplex, thus reinforcing double-strand stability.

The last step in the preliminary computational studies was to substitute all the nucleobases of models **C** and **D** with thymine units. In order to compensate the loss of stabilizing G–C H-bonds, we decided to elongate the duplex with a couple of extra base-pairing units, obtaining a decamer of thymine/adenine pairs. Once again, the integrity of the duplexes was maintained throughout the whole 10 ns simulation (Figure 5, models **E** and **F**). In particular, we considered the fact that the computational studies on model **E** (coincident with the Xu thyminated peptoid oligomer, which was known to hybridise DNA with a T_m of 42 °C)[15] predicted a stable duplex as a propitious auspice for the correct annealing of the designed, carboxylated, congener **F**, which, in silico, behaved in a similar way. Being encouraged by the theoretical outcomes, we promptly moved towards the chemical synthesis of oligomers **6** and **7**.[22]

Chemistry

From a retrosynthetic point of view, we traced target **6** and **7** back to the properly protected dipeptoid unit **8** (Figure 7). The synthon **8** could be easily assembled from an iminodiacetic acid derivative (**9a**) and a 1′-thyminylated ethylglycine residue (**9b**).

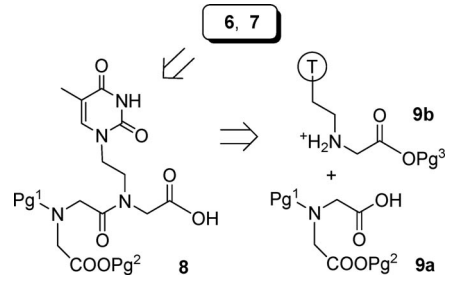
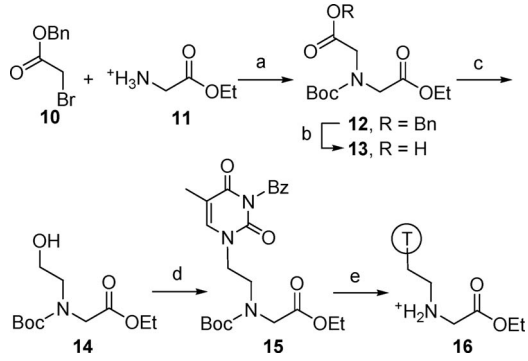


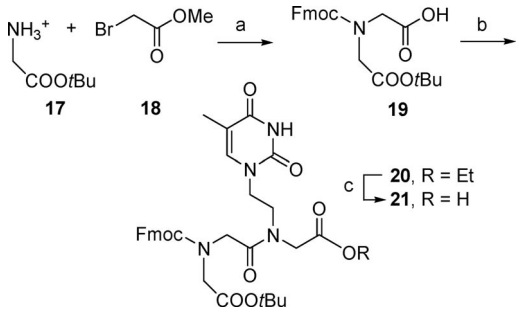
Figure 7. Retrosynthetic scheme for oligomers **6** and **7**. T = thymine. Pg = protecting group.

The synthesis of the monomer started with the elaboration of the required *N*-[2-(thymin-1-yl)ethyl]glycine (**16**), as shown in Scheme 1. We prepared the *N*-deprotected building block in five steps and 26% overall yield. The Mitsunobu reaction[23] of **14** – easily assembled through the reduction of a diprotected iminodiacetic acid – with the known N^3-benzoylthymine[24] gave thyminated adduct **15**. This we deprotected at the two nitrogen atoms in the presence of trifluoroacetic acid to yield α-amino ester **16**.



Scheme 1. Synthesis of *N*-[2-(thymin-1-yl)ethyl]glycine ethyl ester (**16**). Reagents and conditions: (a) (i) DIPEA, DMF, (ii) $(Boc)_2O$, Et_3N, CH_2Cl_2, 88%; (b) H_2, Pd/C, AcOH, quantitative; (c) $BH_3{\cdot}SMe_2$, THF, 69%; (d) N^3-benzoylthymine, DEAD, PPh_3, THF; (e) TFA, CH_2Cl_2, 43%, two steps. T = thymine.

The 2-(7-aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) promoted condensation of **16** with the Fmoc/*t*Bu-protected acid counterpart **19** (Scheme 2) gave the expected dipeptoid **20**. Careful LiOH-mediated hydrolysis preserved the base-labile Fmoc group, affording target dipeptoid monomer **21**.



Scheme 2. Synthesis of *N*-[*N*-(fluoren-9-ylmethoxycarbonyl)-*N*-(*tert*-butoxycarbonylmethyl)glycyl]-*N*-[2-(thymin-1-yl)ethyl]glycine (**21**). Reagents and conditions: (a) (i) DIPEA, DMF, (ii) LiOH, 1,4-dioxane, (iii) FmocCl, $NaHCO_3$, 88%; (b) **16**, HATU, DIPEA, DMF, 95%; (c) LiOH, 1,4-dioxane, 0 °C, 69%.

We manually assembled peptoid oligomers **6** and **7** in a stepwise fashion on a Rink amide solid support using HATU as the coupling agent. We monitored the coupling efficiency by measuring the absorbance of the dibenzofulvene–piperidine adduct (spectroscopically evidenced after every deprotection step). The average coupling yield was higher than 97% (after a double coupling). Once we had condensed all the monomers, we detached the oligomers from the solid support using trifluoroacetic acid. The acid efficiently cleaved also the *t*Bu protecting groups from the carboxy groups. We purified the water-soluble oligomers by HPLC on an anion-exchange column, followed by gel-filtration chromatography on a Sephadex G25 column, yielding desired **6** and **7** as pure compounds (see the Supporting Information). We determined the purity of the synthesised peptoids by HPLC (anion-exchange column) and their identity by MALDI-TOF mass spectrometry.

Hybridisation Studies

In order to verify the ability of peptoids **6** and **7** to bind complementary DNA and/or RNA, we performed UV-monitored melting experiments by mixing the water-soluble, thyminylated oligomers with either $d(A)_{16}$ or poly-r(A), (5 μM peptoid concentration, λ = 260 nm). Our preliminary thermal denaturation analysis of **6** or **7** in the presence of complementary strands of $d(A)_{16}$ or poly-r(A) gave no characteristic S-shaped curve, even after varying the salt conditions and the mixing ratios, demonstrating that no hybridisation occurred in any case (see the Supporting Information). We further confirmed the absence of efficient hybridisation to nucleic acids by CD analysis. In fact, in all the investigated cases, the CD profiles for the mixtures simply resembled the spectra of the polyadenylated, single-stranded DNA or RNA counterparts, indicating no relevant interaction. Additionally, we did not observe the typical diagnostic curve for a duplex → single-strand transition in the CD-monitored thermal denaturation experiments carried out at λ = 260 nm (see the Supporting Information). In addition, we could not observe any signals in the imino region (δ = 10–15 ppm) diagnostic of Watson–Crick base pairings in the ^{1}H NMR spectra of a 1:1 **7**/$d(A)_{16}$ mixture (0.1 mM in each strand), even at low temperatures (see the Supporting Information).

In order to check for intrasystem pairing, we performed annealing experiments by mixing **7** with the (2,4-diamino)-triazin-6-yl-tagged dodecamer **22** (Figure 8).[16] As before, UV melting experiments showed no clear transition from double to single strands upon heating, indicating that no effective binding occurred between the thyminylated strand **7** and triazine-tagged oligomer **22**.

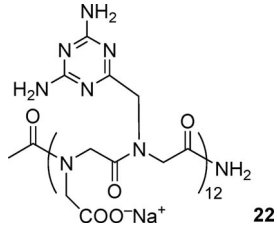


Figure 8. Structure of the dodecamer **22**.

Even though we did not obtain positive evidence of hybridisation to nucleic acids, the current investigation enucleates the limits of the peptoid backbone in the search for novel, biologically active nucleopeptides. The favourable theoretical premise did not consider the loss of entropy due to duplex formation. The delicate balance between enthalpy gain (due to interstrand H-bond formation) and reduction of conformational freedom probably plays against the inter- and intrasystem base pairing. The preliminary theoretical studies were, in fact, executed on a preformed duplex and lacked the dynamic information on possible double-strand formation. The presence of a peptoid backbone is compatible with nucleic-acid hybridisation. This is clearly asserted by the Xu[15] and Eschenmoser[16] contributions. Nonetheless, small backbone/nucleobase variations may have large and unpredictable effects on the nucleosidated peptoid conformation and on binding to nucleic acids. A similar effect has been recently evidenced in the intriguing (and not fully understood) N^{γ}-ε-aminoalkyl-PNA, length-dependent, DNA hybridisation.[19b] In this systematic study, it was found that short, polar side chains (protruding from the N^{γ} atom of peptoid-based PNAs) negatively influenced the hybridisation properties of modified PNAs, whereas longer, polar side chains positively modulated nucleic-acid binding. The reported data did not clarify the reason for this effect, but it seems that factors different from electrostatic interaction are at play.

Conclusions

The present work indicates that the annealing properties of oligoglycine backbones can be sensitive to small structural variations. The substitution of an N^{γ}-methyl group with an N^{γ}-(carboxymethyl) or an N^{γ}-H[14] suppresses nucleic-acid binding, as demonstrated herein [N^{γ}-(carboxymethyl)] and by the work of Almarsson and co-workers (N^{γ}-H).[14] In particular, in our case, we believe that restrictions imposed by a charged oligoglycine backbone and the higher conformational freedom of the nucleobases negatively contributed to the pairing properties of the synthetic nucleopeptoids.

In conclusion, in this paper we have chemically built an orthogonally protected *N*-(carboxymethyl)-*N*-[2-(thymin-1-yl)ethyl] dipeptoid unit. It was efficiently assembled according to standard solid-phase synthesis protocols to give the corresponding octa- and dodecamers. The hybridisation studies indicated that the N^{γ} substitution in a pure peptoid scaffold can play an essential role in the overall annealing process. Whereas thyminylated oligomers with a methyl group linked to the N^{γ}-position bind to their complementary target, other substituents can prevent the double-strand formation. A previous study by Nielsen,[12] reporting the synthesis of a PNA analogue devoid of the tertiary amide carbonyl group, emphasized the importance of the amide group(s) in the PNA for their contribution to backbone rigidity. The present study suggests that overly rigid polar frameworks can hamper duplex formation.

Finally, on the basis of the acquired information, new oligomers with the more dependable *N*-(2-aminoethyl)glycyl backbone (aegPNA), bearing N^{γ} polar chains, will be synthesised and studied with the aim of discovering novel PNA mimics with improved pharmacological traits.

Experimental Section

General Methods: All reactions involving air- or moisture-sensitive reagents were carried out under dry argon or nitrogen by using freshly distilled solvents. Tetrahydrofuran (THF) was distilled from $LiAlH_4$ under argon. Toluene and CH_2Cl_2 were distilled from CaH_2. Glassware was flame-dried (0.05 Torr) prior to use. When necessary, compounds were dried in vacuo over P_2O_5 or by the azeotropic removal of water with toluene under reduced pressure. Starting materials and reagents purchased from commercial suppliers were generally used without purification unless otherwise mentioned. Reaction temperatures were measured externally; reactions were monitored by TLC with Merck silica gel plates (0.25 mm) and visualized by UV light, I_2 or by spraying with H_2SO_4/$Ce(SO_4)_2$, phosphomolybdic acid or ninhydrin solutions and drying. Flash chromatography was performed with Merck silica gel 60 (particle size: 0.040–0.063 mm), and the solvents employed were of analytical grade. Yields refer to chromatographically and spectroscopically (^1H and ^{13}C NMR) pure materials. The NMR spectra were recorded with Bruker DRX 400, (^1H at 400.13 MHz, ^{13}C at 100.03 MHz), Bruker DRX 250 (^1H at 250.13 MHz, ^{13}C at 62.89 MHz) and Bruker DRX 300 (^1H at 300.10 MHz, ^{13}C at 75.50 MHz) spectrometers. ^1H NMR spectra of the duplex **7**/$d(A)_{16}$ were recorded with a Bruker Avance 600 spectrometer. Chemical shifts (δ) are reported in ppm relative to the residual solvent peak ($CHCl_3$: δ = 7.26 ppm; $^{13}CDCl_3$: δ: = 77.0 ppm; CD_2HOD: δ = 3.34 ppm; $^{13}CD_3OD$: δ = 49.0 ppm; $^{13}CCD_2Cl_4$: δ = 72.1 ppm; C_2DHCl_4: δ = 5.80 ppm, or $^{13}CCD_2Cl_4$: δ = 72.1 ppm), and the multiplicity of each signal is designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintuplet; m, multiplet and br., broad. Coupling constants (*J*) are given in Hz. Homonuclear decoupling, COSY-45 and DEPT experiments completed the full assignment of each signal. High-resolution ESI mass spectra were obtained with a Q-Star Applied Biosystem mass spectrometer. ESI-MS analysis in positive-ion mode was performed by using a Finnigan LCQ Deca ion trap mass spectrometer (ThermoFinnigan, San José, CA, USA), and the mass spectra were acquired and processed by using the Xcalibur software provided by Thermo Finnigan. Samples were dissolved in 1:1 CH_3OH/H_2O, 0.1% formic acid and infused into the ESI source by using a syringe pump; the flow rate was 5 μL/min. The capillary voltage was set at 4.0 V, the spray voltage at 5 kV and the tube lens offset at –40 V. The capillary temperature was 220 °C. MALDI-TOF mass spectrometric analyses were performed with a PerSeptive Biosystems Voyager-De Pro MALDI mass spectrometer in the linear mode by using α-cyano-4-hydroxycinnamic acid, picolinic/2-hydroxypicolinic acid or sinapinic acid as the matrix. HPLC analyses were performed with a Beckman System Gold instrument equipped with a UV detector module 166 and a Shimadzu Chromatopac C-R6A integrator. UV measurements were carried out with a Jasco V-530 UV spectrophotometer equipped with a Jasco ETC-505T temperature controller unit. Circular dichroism (CD) experiments were recorded with a JASCO J-715 spectrophotometer equipped with a thermostatically controlled cuvette holder (JASCO PTC-348) in a 0.1 cm path length cuvette. CD spectra were collected from 220 to 300 nm, at 20 nm/min, with a response time of 16 s and at a 1 nm bandwidth. The oligonucleotide $d(A)_{16}$ was assembled with a Millipore Cyclone Plus DNA synthesiser by using commercially available 3′-O-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite 2′-deoxyribonucleosides as building blocks and purified through sequential anion-exchange HPLC/gel filtration chromatography. Polyadenylated ribonucleic acid (60 μM in base) was purchased from Sigma–Aldrich and used as received.

Computational Details: All calculations were performed by using the MACROMODEL 8.5 software package[25] and the AMBER force field.[26] In all geometry-optimization and MD calculations, the GB/SA[21] solvent treatment was used, mimicking the presence of H_2O, for reducing the artifacts derived from the absence of the solvent. The solution-phase NMR structure of 5′-d(GACATAGC)-3′-NH_2-(CTGTATCG)-COOH[17b] (PDB archive code 1PDT) was used as a template to build the models **C**–**F** (Figure 6); the acetyl linker between the base and nitrogen atom of the backbone was replaced by an ethyl linker, and the *N*-(2-aminoethyl) group was converted into a glycine residue. For model **E** and **F**, the sequence was converted into a hybrid $(A\text{-}T)_{10}$ double strand. In particular, the new bases were inserted by maintaining the planarity of the substituted aromatic rings and the H-bond distances for A–T base pairs in the solution-phase NMR structure. The geometries of the peptoid bonds in the strands were built as all-*trans* and used in the subsequent simulations of 10 ns (see below). All built peptoid backbone atoms of models **C**–**F** were optimized by using Polak-Ribiere Conjugate Gradient (PRCG, 9×10^6 steps, convergence threshold 0.001 kJ mol^{-1} Å^{-1}) by fixing the atoms of the DNA strand and the purine/pyrimide bases. The geometries so obtained for the newly built strands underwent MD (100 ps, 300 K) to relax the double helix. After 1 ps of equilibration at 300 K, a 1.5 fs integration time step was used, and 20 structures were collected. Different conformers for structures **C**–**F** were obtained and used for

MD calculations without distance restraints to evaluate the stability of the hybrid duplexes. A temperature of 300 K was used during the dynamics simulations over a period of 10 ns, and a standard constant temperature velocity Verlet algorithm was used to integrate the equation of motions. The SHAKE algorithm[27] was used and applied to all bonds. A 1.5 fs integration time step was used. After 1 ps of equilibration at 300 K, the MD trajectories were obtained by sampling every 40 ps.

Chemistry

12: To a solution of glycine ethyl ester (1.00 g, 7.19 mmol) in DMF (6 mL), DIPEA (2.50 mL, 14.4 mmol) was added, followed by benzyl bromoacetate (0.82 g, 3.60 mmol) 10 min later. The reaction mixture was stirred at room temp. overnight, concentrated in vacuo, dissolved in AcOEt (10 mL) and washed with NaCl/H_2O (1:1). The aqueous phase was extracted with AcOEt (2 × 10 mL). The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated in vacuo, affording a crude material, which was used in the next step without purification. To a solution of the crude material in CH_2Cl_2 (10 mL), Et_3N (0.650 mL, 4.68 mmol) and $(Boc)_2O$ (1.02 g, 4.68 mmol) were added. The reaction mixture was stirred at room temp. overnight, concentrated in vacuo and flash-chromatographed (10–60% diethyl ether in petroleum ether) to give pure **12** as a viscous oil (1.11 g, 88%). ^{1}H NMR (250 MHz, $CDCl_3$, mixture of rotamers): δ = 1.25 and 1.26 (t, J = 7.1 Hz, 3 H, CH_3), 1.37 and 1.42 [s, 9 H, $C(CH_3)_3$], 3.98 and 4.03 (s, 2 H, CH_2NBoc), 4.09 and 4.15 (s, 4 H, CH_2NBoc, CH_2CH$_3$, overlapped), 5.14 and 5.15 (s, 2 H, CH_2Ph), 7.33 (m, 5 H, Ar-H) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 14.1 (br. s), 28.0 (3 C), 49.0, 49.6, 61.0, 66.7, 81.0, 128.3 (2 C), 128.5 (3 C), 135.3, 155.0, 169.6 (2 C) ppm. MS (ESI): m/z (%) = 352.2 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{18}H_{26}NO_6$ 352.1760; found 352.1754.

13: To a solution of **12** (1.86 g, 5.29 mmol) in AcOEt (30 mL), a catalytic amount of 10% Pd/C (50 mg) and AcOH (0.1 mL) were added. The flask was evacuated and flushed with H_2 three times. The reaction mixture was stirred vigorously under H_2 overnight. The mixture was filtered through a short pad of Celite® and concentrated, affording **13** as a viscous oil (1.40 g, quantitative). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ = 1.32 (t, J = 7.1 Hz, 3 H, CH_3), 1.44 and 1.46 [s, 9 H, $C(CH_3)_3$], 3.96 and 4.00 (s, 2 H, CH_2NBoc), 4.10 and 4.11 (s, 2 H, CH_2NBoc), 4.27 (q, J = 7.1 Hz, 2 H, CH_2CH$_3$) ppm. MS (ESI): m/z (%) = 262.1 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{11}H_{20}NO_6$ 262.1291; found 262.1297.

14: To a solution of **13** (1.81 g, 6.93 mmol) in dry THF (35 mL) at 0 °C, BH_3·SMe_2 (13.8 mL, 27.7 mmol, 2 M in THF) was added. The reaction mixture was stirred overnight. The solvent was evaporated under reduced pressure, saturated aq. NH_4Cl was added, and the aqueous layer was extracted three times with CH_2Cl_2 and one time with AcOEt. The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated in vacuo, affording a crude material, which was flash-chromatographed (50–90% diethyl ether in petroleum ether) to give **14** as a viscous oil (1.18 g, 69%). ^{1}H NMR (300 MHz, $CDCl_3$, mixture of rotamers): δ = 1.26 and 1.28 (t, J = 7.1 Hz, 3 H, CH_3), 1.45 and 1.46 [s, 9 H, $C(CH_3)_3$], 3.43 (m, 2 H, HOCH$_2$CH_2NBoc), 3.68 and 3.73 (m, 2 H, HOCH_2CH$_2$NBoc), 3.90 and 3.95 (s, 2 H, OCCH_2NBoc), 4.21 (q, J = 7.1 Hz, 2 H, CH_2CH$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ = 13.9, 14.1, 28.0 and 28.1 [C(CH_3)$_3$], 50.4, 50.9, 51.9, 52.1, 60.8, 61.0, 61.3, 61.4, 80.4 [$C(CH_3)_3$], 155.3, 155.5, 171.6, 171.9 ppm. MS (ESI): m/z (%) = 270.1 (100) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{11}H_{21}NNaO_5$ 270.1317; found 270.1321.

16: To a solution of **14** (0.08 g, 0.32 mmol), N^3-benzoylthymine (0.11 g, 0.48 mmol) and PPh_3 (0.21 g, 0.81 mmol) in dry THF (3 mL) at 0 °C, DEAD (0.13 mL, 0.81 mmol) was added. The reaction mixture was stirred overnight. The solvent was evaporated under reduced pressure, and the crude **15** was dissolved in CH_2Cl_2/TFA (1:3, 2.4 mL) and stirred overnight. Volatiles were removed in vacuo, and the oily residue was precipitated in cold diethyl ether. The white amorphous precipitate was washed again with diethyl ether, dried (high vacuum, room temp., overnight) and used without further purification (36 mg, 43% over two steps). ^{1}H NMR (300 MHz, CD_3OD): δ = 1.35 (t, J = 7.1 Hz, 3 H, CH_3), 1.93 (s, 3 H, CH_3), 3.42 (t, J = 5.8 Hz, 2 H, HNCH_2CH$_2$), 4.03 (s, 2 H, OCCH_2NH), 4.11 (t, J = 5.8 Hz, 2 H, HNCH$_2$CH_2), 4.34 (q, J = 7.1 Hz, 2 H, CH_2CH$_3$), 7.44 (s, 1 H, HC=) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 12.2, 14.3, 46.0, 48.1, 49.0 (CD_3OD, overlapped), 63.6, 112.1, 118.2 (q, J = 290 Hz, CF_3COO^-), 142.4, 153.9, 163.0, (q, J = 38.3 Hz, CF_3COO^-), 166.7, 167.6 ppm. MS (ESI): m/z (%) = 278.1 (100) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{11}H_{17}N_3NaO_4$ 278.1117; found 278.1115.

19: To a solution of glycine tBu ester (6.57, 39.2 mmol) in DMF, (35 mL) DIPEA was added (78.4 mmol, 13.6 mL). The reaction mixture was stirred for 10 min, and methyl bromoacetate (1.86 mL, 19.6 mmol) was added dropwise over 20 min. The reaction mixture was stirred at room temp. for 24 h, concentrated in vacuo, dissolved in AcOEt (10 mL) and washed with NaCl/H_2O (1:1). The aqueous phase was extracted with AcOEt (2 × 10 mL). The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated in vacuo, affording crude **19**, which was used in the next step without purification. To a solution of **19** in 1,4-dioxane at 0 °C, LiOH·H_2O (0.91 g, 21.6 mmol) was added. After the reaction mixture was stirred at 0 °C for 3 h, $NaHCO_3$ (1.8 g, 21.6 mmol) and Fmoc-Cl (5.58 g, 21.6 mmol) were added. The reaction mixture was stirred at room temp. for 24 h, acidified to pH = 3–4 with saturated aq. $NaHSO_4$ and extracted with AcOEt (200 mL × 3). The combined organic phases were washed with H_2O (100 mL × 2) and brine (100 mL), dried (Na_2SO_4), concentrated in vacuo and flash-chromatographed (40% diethyl ether in petroleum ether to 10% methanol in AcOEt, all containing 1% of AcOH) to give **19** as a viscous oil (7.08 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ = 1.48 and 1.49 [s, 9 H, $C(CH_3)_3$], 4.02 and 4.06 (s, 2 H, CH_2NFmoc), 4.08 and 4.19 (s, 2 H, CH_2NFmoc), 4.23 (m, 1 H, CHFmoc), 4.43 (m, 2 H, CH_2Fmoc), 7.31 (m, 2 H, Ar-H), 7.37 (m, 2 H, Ar-H), 7.56 (m, 2 H, Ar-H), 7.75 (m, 2 H, Ar-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, mixture of rotamers): δ = 28.0 (3 C), 46.9 and 47.0 (CHFmoc), 50.4, 50.6 and 51.1 (CH_2NFmoc), 68.5, 68.7 (CH_2Fmoc), 83.2 and 83.7 [$C(CH_3)_3$], 120.0, 125.0, 127.1, 127.8, 141.2, 143.6, 155.9, 169.4, 170.6, 172.4, 173.1 ppm. MS (ESI): m/z (%) = 412.2 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{23}H_{26}NO_6$ 412.1760; found 412.1758.

20: To a solution of **16** (0.15 g, 0.40 mmol) in DMF (3 mL), a solution of **20** (0.32 g, 0.78 mmol), DIPEA (0.260 mL, 1.52 mmol) and HATU (0.29 g, 0.76 mmol) in DMF (3 mL) was added. The reaction mixture was stirred at room temp. overnight, concentrated in vacuo, dissolved in CH_2Cl_2 (16 mL) and washed with saturated aq. NH_4Cl (8 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 16 mL). The combined organic phases were washed with aq. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated in vacuo, affording a crude mixture, which was flash-chromatographed (0–10% methanol in CH_2Cl_2, containing 1% of AcOH) to give **20** as a viscous oil (0.25 g, 95%). ^{1}H NMR (250 MHz, $C_2D_2Cl_4$, 360 K): δ = 1.13 (t, J = 7.1 Hz, 3 H, CH_3), 1.33 [s, 9 H, $C(CH_3)_3$], 1.72 (s, 3 H, CH_3), 3.46 (t, J = 5.8 Hz, 2 H, NCH_2CH$_2$N), 3.70 (t, J = 5.8 Hz, 2 H, NCH$_2$CH_2N), 3.85–4.11 [m, 9 H, CH_2NFmoc (3 C),

C*H*Fmoc, CH_2CH$_3$, overlapped], 4.28 (d, J = 7.0 Hz, 2 H, CH_2Fmoc), 6.90 (s, 1 H, HC=), 7.12–7.27 (m, 4 H, Ar-H), 7.41 (m, 2 H, Ar-H), 7.59 (m, 2 H, Ar-H), 7.82 (br. s, 1 H, N*H*) ppm. ^{13}C NMR (75 MHz, $C_2D_2Cl_4$, 360 K): δ = 10.1, 12.3, 26.4 (3 C), 44.5, 45.6, 45.9, 46.9, 48.2, 49.2, 60.2, 66.6, 80.3, 109.0, 118.2 (2 C), 123.2 (2 C), 125.4 (2 C), 126.0 (2 C), 139.0, 139.5 (2 C), 142.1 (2 C), 148.8, 154.3, 162.3, 167.0 (3 C) ppm. MS (ESI): *m*/*z* (%) = 671.2 (100) [M + Na]$^+$. HRMS (ESI): calcd. for $C_{34}H_{40}N_4NaO_9$ 671.2693; found 671.2691.

21: To a solution of **20** (0.50 g, 0.77 mmol) in 1,4-dioxane (6.5 mL) at 0 °C, LiOH (0.071 g, 1.69 mmol) in H_2O (6.5 mL) was added. The reaction mixture was stirred for 50 min, acidified to pH = 3–4 with saturated aq. $NaHSO_4$ and extracted with CH_2Cl_2 (50 mL × 3) and AcOEt (50 mL × 1). The combined organic phases were washed with H_2O (100 mL × 2) and brine (100 mL), dried (Na_2SO_4), concentrated in vacuo and flash-chromatographed (2–20% methanol in CH_2Cl_2, containing 1% of AcOH) to give **21** as an amorphous white solid (0.33 g, 69%). ^{1}H NMR (250 MHz, $C_2D_2Cl_4$, 360 K): δ = 1.27 [s, 2 H, C(CH_3)$_3$], 1.68 (s, 3 H, CH_3), 3.45–4.28 (m, 7 H NCH_2CH$_2$N, NCH$_2$CH_2N, CH_2NFmoc, C*H*Fmoc, overlapped), 6.90 (s, 1 H, HC=), 7.12–7.27 (m, 4 H, Ar-H), 7.41 (m, 2 H, Ar-H), 7.59 (m, 2 H, Ar-H), 7.81 (br. s, 1 H, N*H*) ppm. ^{13}C NMR (75 MHz, $C_2D_2Cl_4$, 360 K): δ = 10.0, 26.4 (3 C), 44.7, 44.8, 45.6, 47.2 (2 C), 48.3, 66.6, 80.4, 109.3, 118.2 (2 C), 123.2 (2 C), 125.4 (2 C), 126.4 (2 C), 139.1, 139.4 (2 C), 142.1 (2 C), 149.3, 154.4, 162.2, 166.8, 168.0, 168.6 ppm. MS (ESI): *m*/*z* (%) = 643.2 (100) [M + Na]$^+$. HRMS (ESI): calcd. for $C_{32}H_{36}N_4NaO_9$ 643.2380; found 643.2383.

Solid-Phase Synthesis of Oligomers 6 and 7: Linear peptide sequences were synthesised by using standard, manual, Fmoc-based, solid-phase, peptide synthesis protocols. Rink-amide resin (0.075 g, Aldrich, crosslinked with 1% DVB, 100–200 mesh, 0.5–1.5 mmol/g) was swelled in dry *N*-methylpyrrolidinone (NMP, 1 mL) for 20 min and washed once with dry NMP (1 mL). The resin was incubated twice with 20% piperidine/NMP (1 mL) on a shaker platform for 3 min and 7 min respectively, followed by extensive washes with NMP (9 × 3 mL). Compound **21** (0.014 mmol) in dry NMP (0.6 mL), DIPEA (0.028 mmol, 5 μL, 2 equiv.) and HATU (0.014 mmol) were added, and the mixture was agitated on a shaker platform at room temperature for 2 h and then washed with dry NMP (2 × 1 mL). The resin loaded with the first monomer was incubated with a mixture of NMP, 2,6-lutidine and Ac_2O (1.78 mL, 120 μL, 100 μL) on a shaker platform for 30 min and then extensively washed with NMP (6 × 1 mL). The deprotection of the second amino acid was effected as described above. The yields of loading and of the following coupling steps were evaluated by interpolating the absorption of the dibenzofulvene–piperidine adduct (λ_{max} = 301 nm, ε = 7800 M^{-1}cm^{-1}), obtained in the Fmoc deprotection step. After loading of the first monomer, all subsequent *N*-Fmoc monomer-addition and Fmoc-deprotection steps were performed as follows, until the desired oligomer length was obtained. The resin was incubated twice with a solution of *N*-Fmoc-protected monomer (0.052 mmol), HATU (0.050 mmol) and DIPEA (18 μL, 0.010 mmol) in dry NMP (0.6 mL) on a shaker platform for 1 h and then extensively washed with NMP (9 × 1 mL). The chloranil test was performed, and once the coupling was complete, the Fmoc group was deprotected with piperidine as described above, and the resin was washed again to prepare it for the next coupling (the average coupling yield was >97% with double coupling). The oligomer was cleaved from the resin in 1 mL of TFA/*m*-cresol (95:5). The cleavage was performed twice on a shaker platform at room temp. for 30 min The resin was then filtered off, and the filtrates were concentrated under a nitrogen flux. The oily residue was precipitated in cold diethyl ether (30 mL). The white precipitate was washed again with diethyl ether and dried (high vacuum, room temp., overnight).

Purification and Characterization of Oligomers 6 and 7: The crude detached oligomers were analyzed and then purified by HPLC (analytical anion-exchange column; NUCLEOGEN SAX 1000–8/46, 5 μm; linear gradient from 0 to 100% of B solution in A solution over 60 min for the 8-mer **6** and over 30 min for the 12-mer **7**; flowrate of 0.8 mL/min; detection at λ = 260 nm; solution A = 1 mM KH_2PO_4, 20% CH_3CN, pH = 7.0; solution B = 350 mM KH_2PO_4, 1 M KCl, 20% CH_3CN, pH = 7.0). In both cases, the HPLC profiles showed a single major peak at 13.5 min (for **6**) and 11.2 min (for **7**). The corresponding peaks were collected, concentrated and then desalted by size-exclusion chromatography on a Sephadex G25 column eluted with water/ethanol (4:1, v/v). After HPLC purification, 58 OD of pure **6** and 61 OD of **7** could be isolated (OD: optical density unit, equal to the amount of material, which – dissolved in 1.0 mL – gives an absorbance of 1.0 at λ = 260 nm), as determined from UV measurements at λ = 260 nm of the samples dissolved in H_2O at 90 °C. By HPLC analysis (Nucleosil 100-5 C18 Supelco analytical column, 250 × 4.6 mm, 5 μm; linear gradient from 0 to 100% CH_3CN over 30 min in H_2O; flowrate of 0.8 mL/min; detection at λ = 260 nm), the isolated oligomers were >98% pure. MALDI-TOF MS analysis confirmed the expected structures for the two samples, with unique signals at the following *m*/*z* values: **6**: MALDI-TOF MS (positive mode): *m*/*z* (%) = 2610.95 (100) [M + H]$^+$, (70) [M + Na]$^+$. **7**: MALDI-TOF MS (positive mode): *m*/*z* (%) = 3907.30 (100) [M + H]$^+$, (70) [M + Na]$^+$, (40) [M + K]$^+$.

Hybridisation Experiments: Hybridisation experiments were carried out by recording UV- and CD-monitored melting curves at λ = 260 nm of the appropriate solutions of peptoid **6** or **7** mixed with either polyadenyl DNA 16-mer, polyadenyl RNA or the triazine-tagged peptoid **22** (no CD-monitored melting curves were recorded for the last mixture). The UV melting curves were recorded in 1 mL of the test solution in Teflon-stoppered quartz cuvettes of 1 cm optical path length. The concentration of the oligomers was determined spectrophotometrically at λ = 260 nm and 90 °C, by using the following molar extinction coefficients, calculated for the unstacked oligomers: 15400 (A),[28] 8800 (T)[28] and 4200 (2,4-diaminotriazine) cm^{-1}M^{-1}.[16] The melting experiments were performed in two different aq. solutions: solution A: 140 mM NaCl, 10 mM NaH_2PO_4, pH = 7.4 and solution B: 1.0 M NaCl, pH = 7.0. Mixtures of the peptoid **6** or **7** were prepared and analyzed in the following ratios with the complementary DNA, RNA or triazine-tagged oligomer **22**: 1:1 (at approximately 5 μM for each strand), 1:2 (at approximately 5 μM:10 μM) and 2:1 (at approximately 5 μM:2.5 μM), respectively. The resulting solutions were then heated at 85 °C for 10 min and then slowly cooled and maintained at 5 °C overnight. After thermal equilibration at 10 °C, the UV absorption at λ = 260 nm was monitored as a function of the temperature, which increased at a rate of 0.5 °C/min over the range 20–80 °C. For all the mixtures prepared as described above, CD spectra were then recorded at 20 °C in a 0.1 cm path length cuvette. The wavelength was varied from 220 to 340 nm at 20 nm min^{-1}. CD spectra were recorded with a response of 16 s at a 2.0 nm bandwidth and normalised by subtraction of the background scan of buffer alone. The molar ellipticity was calculated from the equation $[\vartheta] = \vartheta/c\,l$ where ϑ is the relative intensity, c the concentration of the oligomer and l is the path length of the cell in cm.

Supporting Information (see footnote on the first page of this article): Computational details, purification of oligomer **6** and **7**,

UV-monitored melting curves of **7** mixed with $d(A)_{16}$, poly-r(A) and (2,4-diamino)triazin-6-yl-tagged dodecamer **22**, CD spectra for **7** mixed with $d(A)_{16}$ and ^1H NMR spectra (600 MHz) of **7** mixed with $d(A)_{16}$ in a 1:1 ratio at 0.1 mM in each strand.

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