0 °C and then stirred for 1 h at room temperature. At −196 °C dichlorosilane (5.7 g, 56 mmol) was condensed onto the mixture, which was then stirred for 1 h at −78 °C. After the mixture was warmed to room temperature all volatile components were separated from the salt by condensation. $H_2Si(ONMe_2)_2$ (4.2 g, 28 mmol, 50%) could be isolated at −35 °C as an air-sensitive, colorless liquid (m.p. 0 °C) by condensation through a series of traps (−35, −35, −78, and −196 °C). ^1H NMR: $\delta = 2.42$ (s, 12H, H_3C), 4.70 (s, 2H, H_2Si); ^{13}C NMR: $\delta = 49.7$ (qq, $^1J(C,H) = 135.4$ Hz, $^3J(C,H) = 5.4$ Hz, CH_3); $^{15}N\{^1H\}$ NMR (DEPT): $\delta = -249.2$ (s); $^{17}O\{^1H\}$ NMR: $\delta = 141.1$ (s); ^{29}Si NMR (DEPT): $\delta = -49.8$ (q, $^1J(Si,H) = 219.3$ Hz); IR (gas): $\tilde{\nu} = 2193$ cm^{-1} (s, ν(Si–H)); MS (CI): $m/z = 149$ [$M^+ - 1$].

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PHONA–PNA Co-Oligomers: Nucleic Acid Mimetics with Interesting Properties

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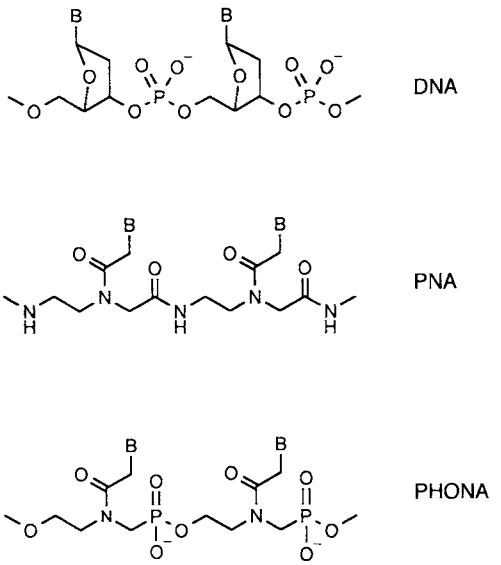
Oligonucleotide analogues are of special interest in molecular biology and medicinal chemistry because of their use as diagnostic agents and their potential application as therapeutics.[1,2] PNAs are nucleic acid mimetics in which the sugar–phosphate backbone is completely replaced by an *N*-(2-aminoethyl)glycine scaffold. The nucleobases are attached to this backbone through methylenecarbonyl linkers (Scheme 1).[3–5] PNAs bind remarkably strongly to complementary DNA and RNA sequences.[5] This is partly due to the



Scheme 1. Structures of DNA, PNA, and PHONA.

lack of negative charges on the PNA backbone, which, however, results in poor water solubility and inadequate cellular uptake.[5,6] The reintroduction of negative charges into PNAs is possible by, for example, the synthesis of PNA–DNA chimeras,[7] or by the synthesis of a further structural variant, the PHONAs (Scheme 1).[8] PHONAs are analogues of PNAs in which the peptide bond is replaced by a phosphonic acid ester bridge.

The abundance of available nucleic acid mimetic structures offers the organic chemist the possibility of combining appropriate building blocks to create oligomers with customized properties. On the other hand prediction of the binding properties of chimeric oligomers is fraught with uncertainty, as the structures of the complexes of different nucleic acid analogues with their complementary DNA can vary widely. This is illustrated by a comparison of the structures of DNA · DNA and PNA · DNA duplexes.[9] Here we report the synthesis and binding properties of alternating PHONA–PNA

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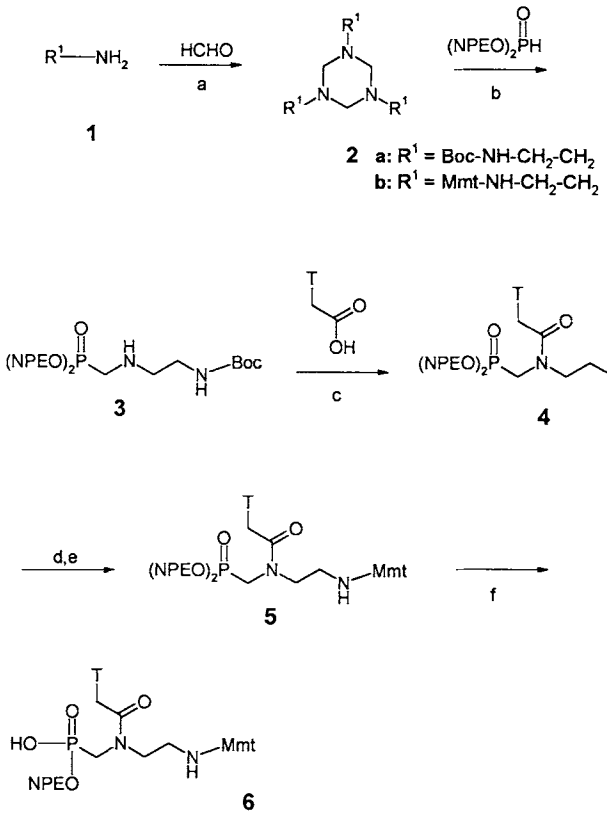
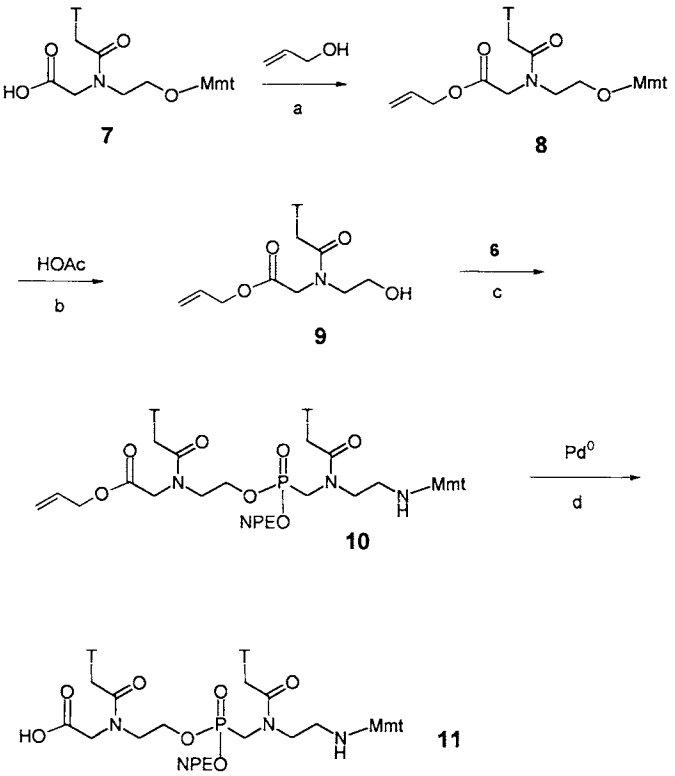
oligomers. The oligomers contain only the nucleobase thymine, as protection of exocyclic amino functions is unnecessary.

The central building block for the synthesis of PHONA–PNA oligomers is the dimer block **11** (see Scheme 3), which carries two orthogonal protecting groups: the acid-labile 4-methoxytriphenylmethyl (Mmt) protecting group for the amino function and the 4-nitrophenylethyl (NPE) phosphonic acid ester,[10] which can be cleaved by β-elimination. The synthesis of the amino-PHONA building block is shown in Scheme 2. The strategy is largely analogous to the previously



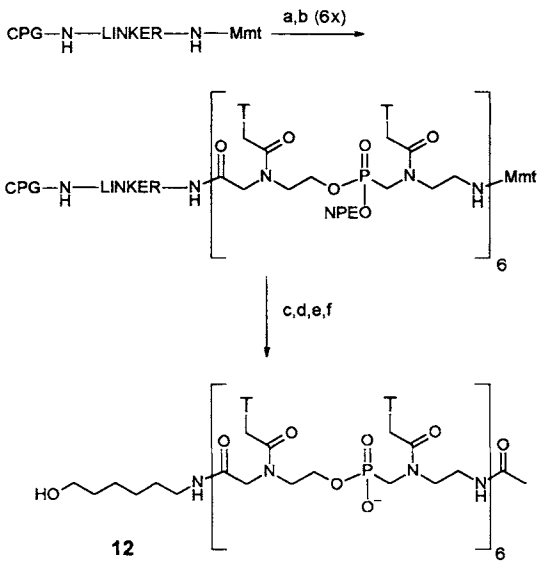
Scheme 2. Synthesis of the PHONA monomer; T = 1-thyminyl: a) 1.5 equiv HCHO, methanol, room temperature (RT), 1 h, 37%; b) 1 equiv phosphite, 2.5 h, 100°C, 23%; c) 1 equiv 1-thyminylacetic acid, 3 equiv *N*-ethylmorpholine, 1.1 equiv HATU, DMF, 24 h, RT, 66%; d) 100% trifluoroacetic acid, 0–10°C, 100%; e) 1.1 equiv MmtCl, 3 equiv diisopropylethylamine, 12 h, 0–5°C, 100%; f) 0.1M diazabicycloundecene (DBU) in acetonitrile, 24 h, 0°C, 77%.

described synthesis of the corresponding hydroxy-PHONA building block.[8] However, it was necessary to use the *tert*-butyloxycarbonyl (Boc) protecting group for the amino function. This could be exchanged for the Mmt group after attachment of the phosphonic acid. The addition of the phosphite to the corresponding Mmt-protected imine **2b** did not give the desired phosphonic acid, presumably because partially released formaldehyde reacted with both secondary amino functions. The hydroxyethylglycine-based building block **7** [7a,b] (Scheme 3) was converted into its allyl ester, and after cleavage of the Mmt protecting group, was coupled with PHONA monomer **6** by using the coupling reagent 3-nitro-1-(*p*-toluenesulfonyl)-1H-1,2,4-triazole (TSNT).[11] The allyl ester of dimer block **10** can be cleaved selectively[12] with Pd^0, which leaves the Mmt and NPE protecting groups intact.



Scheme 3. Synthesis of mixed PHONA–PNA building block **11**: a) 2 equiv allyl alcohol, 3 equiv TSNT, pyridine, 24 h, 0°C, 86%; b) 80% acetic acid, 3 h, RT, 100%; c) 1 equiv **6**, pyridine, 4 equiv TSNT, 14 h, 0°C, 65%; d) $Pd[\{PPh_3\}]_4$, PPh_3, diethylammonium hydrogen carbonate, CH_2Cl_2, 1 h, 70%.

The assembly of the oligomer was carried out on aminohexylsuccinyl–CPG solid support analogously to PNA synthesis.[7f] The synthesis cycle (Scheme 4) consists of



Scheme 4. Synthesis of alternating PHONA–PNA oligomer **12**: LINKER: $-C(O)CH_2CH_2C(O)O(CH_2)_6-$; a) 80% acetic acid, 3 h, 100%; b) double coupling with 4 equiv **11** (0.2M solution in DMF), 8 equiv *N*-ethylmorpholine, 4 equiv HOAt (1-hydroxy-7-azabenzotriazole), 4 equiv HATU [13], 5 h, RT; 90%; c) 80% acetic acid, 3 h, 100%; d) acetic anhydride, *N*-methylimidazole, 10 min, RT; e) 0.5M DBU in acetonitrile, 6 h, RT; f) conc. NH_4OH, 12 h, RT. MS (electrospray): M_{calcd} for $C_{134}H_{185}N_{43}O_{62}P$: 3576.04; M_{found}: 3575.71.

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removal of the Mmt group with 80% acetic acid and coupling of the dimer block **11**. The use of a fourfold excess of **11** and HATU as the coupling reagent[13] resulted in coupling efficiencies of greater than 90%. After completion of oligomer assembly the N-terminus was acetylated with acetic anhydride, and the NPE groups were removed by treatment with 0.5 M DBU in acetonitrile. The oligomer **12** was then cleaved from the solid support by treatment with concentrated NH_4OH and purified by polyacrylamide gel electrophoresis. The highly water-soluble oligomer **12** was characterized by HPLC and electrospray-MS; its mass is 3575.7 (calculated: 3576.0).

The binding properties of alternating PHONA–PNA co-oligomer **12** were investigated by hybridization with complementary $(dA)_{12}$. On heating the hybrid gives a clear phase transition (Figure 1), with a melting temperature of 62.6°C.

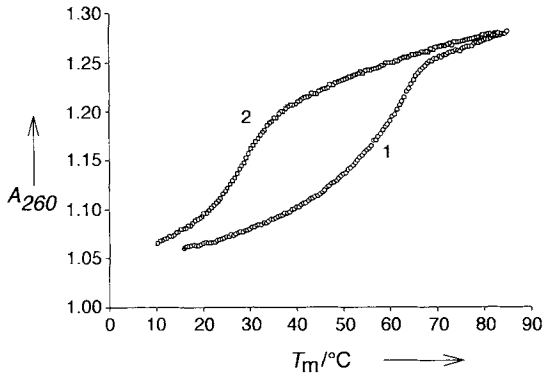

Figure 1. Determination of the melting temperature of the hybrid formed by $d(A)_{12}$ and **12** in the ratio 1:1 by UV spectroscopy at 270 nm. Buffer: 140 mM KCl, 10 mM NaH_2PO_4, 0.1 M EDTA, pH = 7.4; heating and cooling rate: 1 K min^{-1}.

As with PNAs, however, the cooling curve displays a marked hysteresis, with a melting temperature of 29.0°C. The sequence specificity of the binding is clearly demonstrated by the drastic drop in melting temperature on introduction of a mismatch in the complementary DNA strand. Replacement of just one (dA) by (dG) to give **14** results in a drop in T_m to 52.3°C. Replacement of two neighboring (dA)'s by $(dG)_2$ to give **15** gives twice as large a drop in T_m to 39.5°C, and replacement of two nonneighboring (dA)'s to give **16** reduces the T_m to 36.9°C. In this case no phase transition could be observed on cooling. Under the same conditions the corresponding DNA · DNA duplex had a melting temperature of 29.3°C (Table 1).

Comparison with the UV-melting properties of DNA · DNA and PNA · DNA complexes (the T_m of the complex of PNA-T_{10} with $(dA)_{10}$ is 73°C[14]) indicates that PHONA–PNA co-oligomers resemble the latter more closely. The prediction that in this case, as with PNAs, triple-helical

Table 1. Melting temperatures T_m of **12** with completely or partially complementary DNA (ratio 1:1; buffer 140 mM KCl, 10 mM NaH_2PO_4, 0.1 M EDTA, pH = 7.4).

Strand 1	Strand 2	T_m[°C][a]	T_m[°C][b]
$(dA)_{12}$ **13**	**12**	62.6	29.0
$(dA)_6dG(dA)_5$ **14**	**12**	52.3	17.1
$(dA)_5(dG)_2(dA)_5$ **15**	**12**	39.5	15.0
$(dA)_3dG(dA)_3dG(dA)_4$ **16**	**12**	36.9	–
$(dA)_{12}$ **13**	$(dT)_{12}$	29.3	29.0

[a] On heating. [b] On cooling.

structures are formed was confirmed by measurement of a Job plot.[15] (Figure 2; 2:1 ratio of **12** to $(dA)_{12}$). In addition the UV-melting behavior of a 1:1 and a 2:1 ratio of **12** to $(dA)_{12}$ are identical, the only difference being a doubling of the

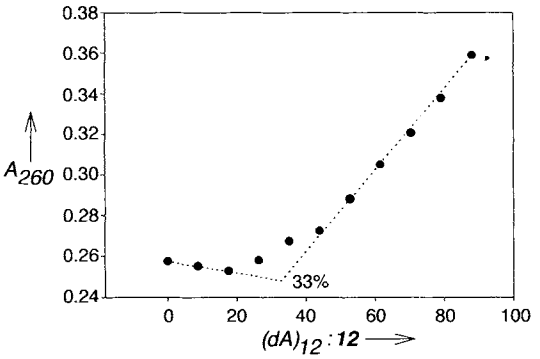

Figure 2. Job plot (continuous titration) of the interaction between **12** and $d(A)_{12}$: UV absorption at 260 nm versus mole ratio $d(A)_{12}$/**12** at 20°C, 500 mM NaCl, 10 mM HEPES, pH 7.5; 2 h equilibration before each measurement.

change in absorbance at the phase transition. Presumably half of the $(dA)_{12}$ present is bound in a $(\mathbf{12})_2 \cdot (dA)_{12}$ complex, and the other half is unbound. The hysteresis observed on cooling is possibly due to formation of the $\mathbf{12} \cdot (dA)_{12}$ duplex, which would suggest relatively slow kinetics for the formation of the triple helix.[16]

The high melting temperature of 62.6°C shows that the PHONA–PNA transitions cause very little structural perturbation in the triple helix. The somewhat lower melting temperature relative to that of $(PNA)_2 \cdot DNA$ triple helices is easily explained by the repulsion of the negative charges of the phosphate and phosphonate groups. These charges reverse the dependence of the melting temperature on salt concentration that is observed for PNAs. Changing the buffer salt concentration from 140 mM KCl to 500 mM NaCl had very little effect on the melting temperature (T_m = 64.7°C).

We have described the synthesis of new nucleic acid mimetics, the alternating PHONA–PNA co-oligomers. Their binding properties with complementary DNA are very similar to those of PNAs, but they have a much better water solubility. The synthesis and binding properties of mixed sequences, as well as the evaluation of cellular uptake and stability under biological conditions, will be reported separately.

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Cylindrical Molecular Brushes**

Peter Dziezok, Sergei S. Sheiko, Karl Fischer, Manfred Schmidt,* and Martin Möller

Regular brush- or comblike macromolecules are principally accessible by homopolymerization of macromonomers. Until recently, however, the degree of polymerization of the main chain did not significantly exceed the length of the macromonomer itself, and almost spherical or starlike structures were obtained. The work presented here was initiated by a report from Tsukahara et al., who successfully polymerized methacryloyl end functionalized polystyrene macromonomers with extremely high degrees of polymerization ($P_w \approx 1000$).[1] The subsequent characterization clearly demonstrated that such molecules adopt the structure of a cylindrical brush with an extremely stiff main chain and a Kuhn statistical segment length of $l_k \approx 1000-2000$ Å.[2] The stiffness of the normally flexible polymethacrylic main chain originates from overcrowding of the side chains; a polystyrene chain with a molar mass of 2000–4000 g mol^{-1} is attached to the main chain every 2.5 Å.[3–5]

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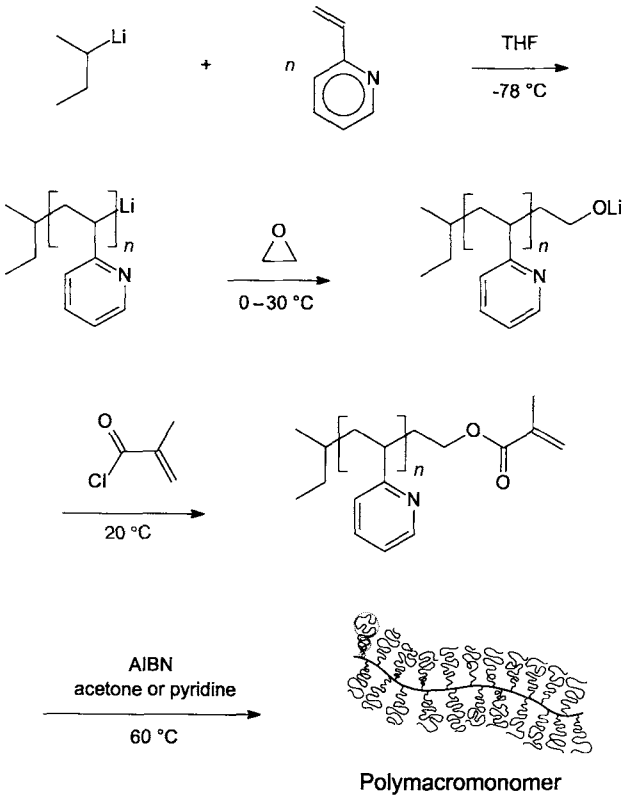
We report here on the synthesis and structure of a polymacromonomer consisting of poly-2-vinylpyridine (PVP). Compared to polystyrene (PS), polyvinylpyridine has many advantages: It is easily converted into a polyelectrolyte brush, complexed by heavy metal ions, and undergoes strong interactions with various polar substrates. The PVP macromonomer was prepared according to Scheme 1. The



Scheme 1. Synthesis of the PVP polymacromonomer; AIBN = azobis(isobutyronitrile).

success of the synthesis was monitored by matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Figure 1). Free radical polymerization

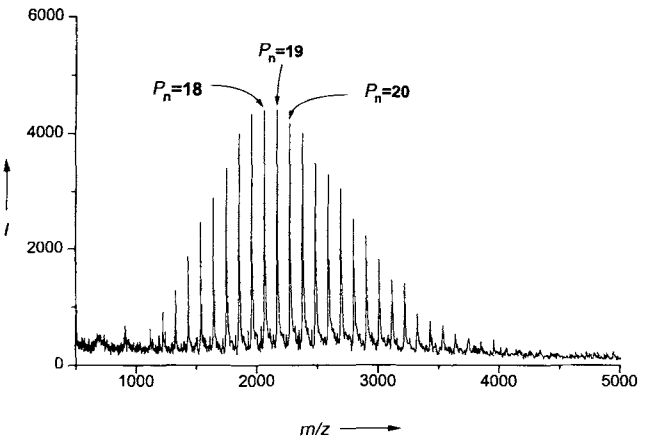


Figure 1. MALDI-TOF mass spectrum of a PVP macromonomer (intensity I in arbitrary units). The signals indicate different degrees of polymerization (P_n). The molar masses for the individual peaks are within ±0.5 Dalton of the expected molar masses of the end-functionalized PVP.

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