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Abstract: The two main branches of bionanotechnology involve the self-assembly of either peptides or DNA. Peptide scaffolds offer chemical versatility, architectural flexibility and structural complexity, but they lack the precise base pairing and molecular recognition available with nucleic acid assemblies. Here, inspired by the ability of aromatic dipeptides to form ordered nanostructures with unique physical properties, we explore the assembly of peptide nucleic acids (PNAs), which are short DNA mimics that have an amide backbone. All 16 combinations of the very short di-PNA building blocks were synthesized and assayed for their ability to self-associate. Only three guanine-containing di-PNAs—CG, GC and GG—could form ordered assemblies, as observed by electron microscopy, and these di-PNAs efficiently assembled into discrete architectures within a few minutes. The X-ray crystal structure of the GC di-PNA showed the occurrence of both stacking interactions and Watson–Crick base pairing. The assemblies were also found to exhibit optical properties including voltage-dependent electroluminescence and wide-range excitation-dependent fluorescence in the visible region.