Supporting Information

A Method for Solid-Phase Synthesis of Oligonucleotide 5'-Peptide-Conjugates Using Acid-Labile α-Amino Protections

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Experimental

General Methods. Amino acids derivatives were purchased from Novabiochem, Bachem, or Fluka, and dried under reduced pressure over P_2O_5. N^{α}-Ddz- and N^{α}-Bpoc-amino acids either purchased or prepared as dicyclohexylammonium (DCHA) or cyclohexylammonium (CHA) salts were quantitatively recovered as free acids according to Merrifield[1] prior to synthesis, and used after overnight drying under low pressure in the presence of a desiccant. Short-term storage (up to a month or longer) of the N^{α}-Bpoc-amino acids as free acids was also possible without detectable decomposition, if kept cold and dry. Phosphoramidites, pre-loaded solid-supports, ancillary reagents, and solvents for DNA and peptide synthesis were purchased from Applied Biosystems or Glen Research. Solvents were dried over molecular sieves and blanketed with argon. Reversed-phase chromatography was accomplished on a Jasco apparatus equipped with a Jones Genesis C18 column (3 × 150 mm). A linear gradient (0–12%, 46 min) of acetonitrile in 50 mM triethylammonium acetate (pH 6.5) was applied at 50 °C, with a flow rate of 0.43 mL/min. The UV detection was carried out at 260 nm. Ion-exchange chromatography was performed on a Jasco apparatus equipped with a Dionex Nucleopac PA-100 column (4 × 250 mm). A linear gradient (5–45%, 78 min) of 0.375 M $LiClO_4$ in 10 mM Tris-Cl (pH 8.3) – 30% acetonitrile was applied at 50 °C, with a flow rate of 1 mL/min. The UV detection was carried out at 260 nm. NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer at 400 and 100 MHz for ^{1}H and ^{13}C, respectively. ^{1}H and ^{13}C chemical shifts are reported in ppm downfield from tetramethylsilane and were determined using residual non-deuterated solvent as an internal standard. Mass analysis was performed on a Micromass LCT ESI–TOF mass spectrometer using leucine enkephalin as an internal mass standard. Conjugates were analyzed in negative mode as solutions in acetonitrile/water, 1:1 (v/v). Molecular weights of the conjugates were reconstructed from the *m/z* values of the multiply deprotonated molecules using the mass deconvolution program of the instrument's MassLynx software package.

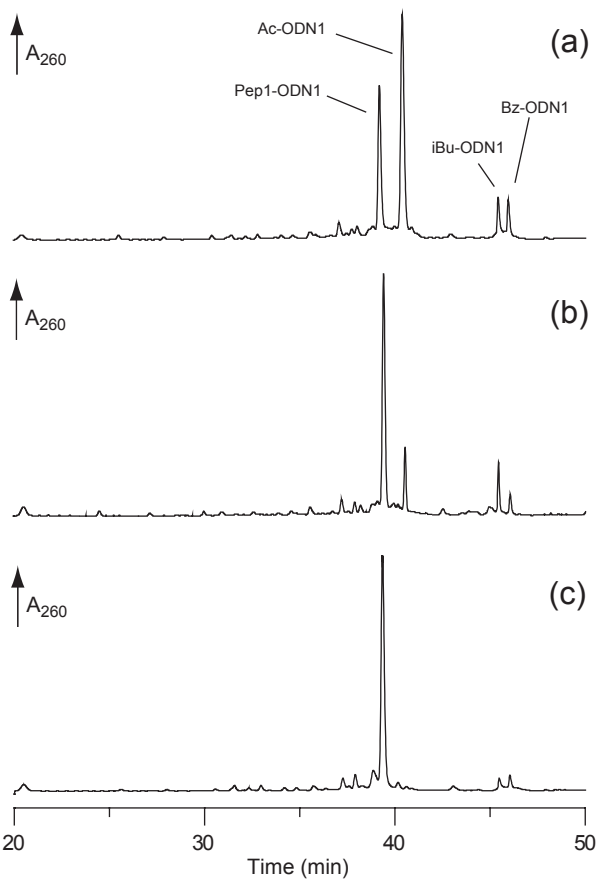

Figure S1. HPLC traces of crude **Pep1-ODN1** synthesized by (a) following the method of entry 2, Table 2; (b) following the method of entry 4, Table 2; and (c) following method in entry 6, Table 2. RP HPLC analysis was carried out on a Jones Genesis AQ C18 column (3 × 150 mm) with elution by a linear gradient (0–12%, 46 min) of acetonitrile in 50 mM triethylammonium acetate (pH 6.5), with a flow rate of 0.43 mL/min. Temperature was set at 50 °C and UV monitoring was performed at 260 nm.

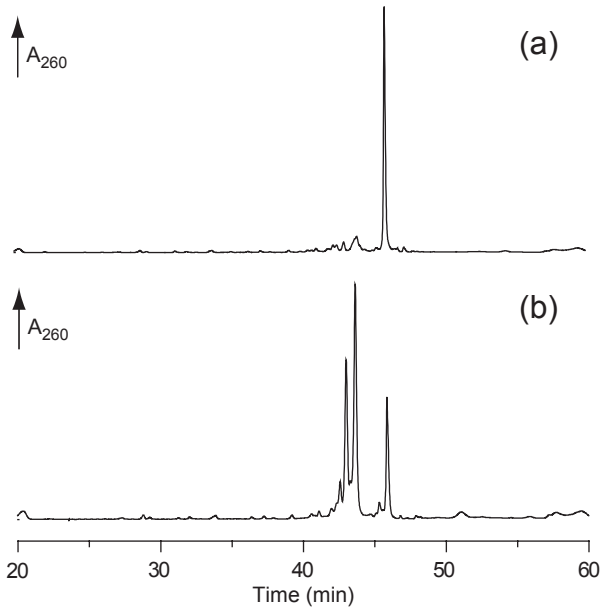

Figure S2. RP HPLC traces of crude **Pep2-ODN2** products synthesized (a) by Bpoc-chemistry and (b) by Fmoc-chemistry. Reversed-phase chromatography was carried out on a Jones Genesis AQ C18 column (3 × 150 mm) with elution by a linear gradient (0–12%, 46 min) of acetonitrile in 50 mM triethylammonium acetate (pH 6.5), with a flow rate of 0.43 mL/min. Temperature was set at 50 °C and UV monitoring was performed at 260 nm.

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[1] Feinberg, R. S.; Merrifield, R. B. *Tetrahedron* **1972**, *28*, 5865–5871.