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The Solid Phase Synthesis of *N*-Alkylcarbamate Oligomers

Sari J. Paikoff, Troy E. Wilson, Charles Y. Cho, Peter G. Schultz*

Howard Hughes Medical Institute
Department of Chemistry
University of California
Berkeley, CA 94720

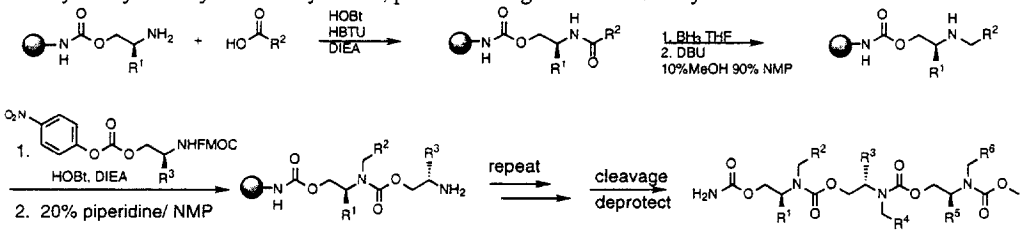
Abstract: An efficient method for the solid phase synthesis of *N*-alkylcarbamate oligomers from alternating carboxylic acid and N^{α}-Fmoc protected chiral *p*-nitrophenylcarbonate monomers has been developed. The general synthetic scheme involves four steps per coupling cycle: deprotection of the terminal amino group of the growing oligomer, acylation of the free amine with a carboxylic acid monomer, reduction of the resulting amide bond with borane and coupling of the secondary amine to a N^{α}-Fmoc protected *p*-nitrophenyl carbonate monomer. This novel biopolymer which has two side chain residues per backbone carbamate linkage and no backbone hydrogen bond donors may provide new frameworks for drug design as well as folded domains with novel physical and biological properties. Copyright © 1996 Elsevier Science Ltd

Polypeptides can fold into a large number of three dimensional architectures with functions ranging from selective binding and catalysis to cellular structure and motility. The remarkable properties of polypeptides suggest that synthetic polymers of defined lengths and sequence composed of unnatural building blocks may also possess novel chemical and biological properties.[1-5] Such "unnatural biopolymers" may afford improved frameworks for drug design as well as new folding patterns which may serve to test current notions of polypeptide structure and folding. In order to begin to characterize polymers of this sort, efficient biosynthetic or solid-phase synthetic routes must be developed. Reported here is a method for the solid-phase synthesis of oligo(*N*-alkylcarbamates) from alternating chiral amino alcohol and carboxylic acid building blocks.

Previously, it was shown that oligocarbamates (and libraries thereof) could be synthesized by solid phase methods from optically active N^{α}-Fmoc protected-*p*-nitrophenyl carbonate monomers and that these oligocarbamates are more hydrophobic and protease resistant than oligopeptides.[1] Here we report conditions which allow for the selective alkylation of the main-chain carbamate nitrogen atoms. This modification simultaneously increases the density of side-chains, removes main-chain hydrogen bond donors and decreases the conformational freedom of the backbone.[6] The general scheme for the solid phase synthesis of this oligomer involves four steps per cycle: deprotection of the terminal amino group of the growing oligocarbamate chain, acylation of the free amine with a carboxylic acid monomer, reduction of the amide bond with borane and coupling of the resulting secondary amine to the next N^{α}-Fmoc protected *p*-nitrophenylcarbonate monomer. Previously, reductive amination conditions have been used to effect nitrogen alkylations on solid support.[7] In contrast, the introduction of a functional group on nitrogen by amine acylation allows the use of a large number of carboxylic acid monomers and takes advantage of existing efficient methods for solid phase amide bond formation.[8] Furthermore, borane will selectively reduce amide linkages in the presence of carbamates and is tolerant of most acid-labile side-chain protecting groups.[9,10]

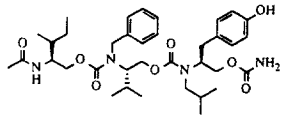
Oligomer synthesis was carried out on Rink resin,[11] which is stable both to borane reduction and to the basic conditions required to hydrolyze the resulting nitrogen-boron complex. Solid-phase synthesis was

initiated by coupling an N^{α}-Fmoc protected *p*-nitrophenylcarbonate monomer (0.3 mmol), derived from the corresponding optically active amino alcohol, to the free amine of Rink resin (0.1 mmol free amine, 0.46 mmol/g) in *N*-methylpyrrolidinone (NMP) in the presence of *N*-hydroxybenzotriazole (HOBt, 1.0 mmol) and diisopropylethylamine (DIEA, 0.3 mmol).[1a,b] The Fmoc protecting group was removed by treatment with 20% piperidine in NMP. Acylation of the primary amine was carried out by addition of the desired carboxylic acid (1.0 mmol), HOBt (1.0 mmol), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 1.0 mmol) and DIEA (6 mmol) in 3 mL DMF at room temperature for 1 h followed by washing of the resin with CH_2Cl_2. The Kaiser ninhydrin test[12] indicated quantitative coupling efficiencies as did cleavage of the products from solid support and high performance liquid chromatography (HPLC) analysis of reaction products. The resin was then suspended in a 1.0 M solution of borane in tetrahydrofuran (THF) at 50°C for 1 h followed by careful quenching of the excess reagent with methanol. While borane reductions of amide bonds have been worked up under both acidic and alkaline conditions, the linker and side-chain protecting groups necessitated an alkaline work up procedure. Optimal results were achieved by the addition of three equivalents of a 0.06 M solution of 1,8- diazabicyclo[5.4.0]undec-7-ene (DBU) in NMP:methanol (9:1) at room temperature for 6 hr, followed by extensive washing of the resin with CH_2Cl_2. The reduction was monitored by HPLC analysis of the cleaved product and the reduction typically proceeded in quantitative yield. The next N^{α}-Fmoc protected *p*-nitrophenylcarbonate monomer (0.5 mmol) was then coupled to the support-bound secondary amine in THF with HOBt (1.0 mmol) and DIEA (1.1 mmol) at 50 °C; after 5 hr the resin was resubmitted to the coupling conditions. Ninhydrin test,[12] bromophenol blue staining[13] and quantitative Fmoc analysis[14] indicated that even difficult couplings, such as the coupling of a valyl *p*-nitrophenyl carbonate monomer to a sterically bulky isobutyl secondary amine, proceeded in greater than 96% yield.

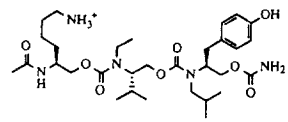


Scheme 1

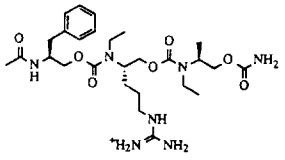
Cleavage of the oligomers from the support and complete side-chain deprotection was accomplished by a low/high [15] cleavage procedure using 10% trifluoroacetic acid (TFA) in CH_2Cl_2 followed by treatment with 90% aqueous TFA.[15] Following removal of TFA *in vacuo*, crude material was dissolved in ethyl acetate and precipitated by addition to a 95:5 hexane: *t*-butylmethyl ether solution followed by repeated washing of the precipitate with the same solvent. Using these methods, four pentamers were synthesized.



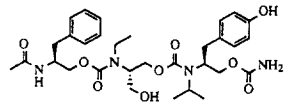
1: Ac ^{t}F^{n}V^{c}L^{n}Y^{c} (68%)



2: AcK^{c}Et^{n}V^{c}L^{n}Y^{c} (80%)



3: AcF^{c}Et^{n}R^{c}Et^{n}A^{c} (72%)



4: Ac F^{c}Et^{n}S^{c}V^{n}Y^{c} (73%)

Structures of oligomers were confirmed by NMR and mass spectroscopy of the purified oligomers. In general, the *N*-alkyl carbamate oligomers could be isolated in reasonably high yield (70-90%) and high purity.[18] The synthetic methods are compatible with a variety of side chain residues (amino, guanidine, hydroxylic, etc.). Evaluation of the structural and pharmacological properties of oligo(*N*-alkylcarbamates) is currently being undertaken.

Acknowledgement. This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Division of Material Sciences, and also by the Division of Energy Biosciences of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098 and from the Office of Naval Research (Grant no. N00014-93-1-0380). PGS is an Howard Hughes Medical Institute Investigator. TEW thanks the Howard Hughes Medical Institute and the Fannie and John Hertz Foundation for predoctoral fellowships and CYC thanks the Glaxo Research Institute and Eli Lilly and Company for predoctoral fellowships. We are grateful to Dr. Edmund Moran and Sara Cherry for the synthesis of some of the monomers used in this work.

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16. Analytical HPLC was performed on a Rainin UV-1 system with a Rainin Instruments Microsorb C-18 column (4.6 x 250 mm, 5 μm particle size) with a gradient elution (solvent A H_2O/0.1% TFA, solvent B CH_3CN/0.08% TFA) of 5-100% B over 60 min.
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18. Spectroscopic data for the *N*-alkyl carbamates synthesized on solid support is as follows:
    ***N*-alkylcarbamate 1**. Cleavage of 0.024 mmol of resin gave 11 mg (68%). ^{1}H NMR (400 MHz, DMSO-d_6 373 K) 0.60-1.10 (m, 18), 1.24-1.30 (m, 4), 1.4-1.6 (m, 1), 1.78 (s, 3), 1.9-2.0 (m, 2), 2.6-3.0 (m, 4H), 3.8-4.5 (m, 9), 5.9-6.1 (2, br), 6.65 (d, 2, J=8.2), 6.95 (d, 2, J=8.4), 7.10-7.30 (m, 3), 7.45 (m, 1), 7.81 (m, 1). HRMS (FAB): Calcd. for $C_{36}H_{55}N_4O_8$: 671.4020. Found: 617.4020.
    ***N*-alkylcarbamate 2**. Cleavage of 0.034 mmol of resin gave 20.1 mg (80%). ^{1}H NMR (400MHz, DMSO-d_6, 373 K). 0.67 (d, 3, J=6.5), 0.69 (d, 3, J=6.5), 0.94 (m, 6), 1.10 (t, 3, J=6.9), 1.13 (s, 3), 1.15 (m, 2), 1.20-1.70 (m, 10), 2.75 (m, 2), 3.64-4.21 (m, 9), 6.10-6.14 (br, 2), 6.72 (d, 2, J= 8.1), 6.94 (d, 2, J=8.2), 7.35 (br, 3), 7.54 (br, 1). HRMS (FAB): Calcd. for $C_{31}H_{55}N_5O_8$: 624.3972. Found: 624.3968
    ***N*-alkylcarbamate 3**. Cleavage of 0.024 mmol of resin gave 12 mg (72%). ^{1}H NMR (400MHz, DMSO-d_6, 373K), 1.02-1.11 (m, 13), 1.43-1.47 (m, 4), 1.74 (s, 3), 2.73 (m, 2), 3.04 (m, 2), 3.93-4.41 (m, 9), 6.14 (br, 2), 6.89 (br, 2), 7.23 (m, 5), 7.43 (br, 1). HRMS (FAB): Calcd for $C_{27}H_{45}N_7O_7$: 580.3459. Found: 580.3452.
    ***N*-alkylcarbamate 4**. Cleavage of 0.020 mmol of resin gave 9 mg (73%). ^{1}H NMR (400 MHz, DMSO-d_6, 373 K) 0.75 (t, 3, J=6.5), 0.94 (d, 3, J=6.6), 1.12 (d, 3, J=6.9), 1.43 (m, 5); 1.81 (s, 3), 2.81-2.93 (m, 4), 3.24-4.21 (m, 9), 6.02 (br, 2), 6.63 (d, 2, J=8.4), 6.93 (d, 2, J=8.4), 7.23 (m, 3), 7.32 (2, m). HRMS (FAB): Calcd. for $C_{31}H_{45}N_4O_9$: 617.3186. Found: 617.3180.

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