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Hydrophilic Polymers Containing Chiral Nucleic Acid Base Pendants as Polynucleotide Analogs

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Dedicated to Prof. Dragutin Fleš on the occasion of his 60th birthday

SUMMARY

A survey of our recent work on synthetic polynucleotide analogs is given. Propionic acid and 3-methyl butyric acid derivatives substituted in the 2-position with nucleic acid bases have been used as chiral pendants for attachment to hydrophilic polyamine backbones. Hindered rotation about the amide bonds formed promotes a base-stacked structure as shown by ultraviolet hypochromic effects versus model compounds. If the pendant has been resolved, an optically active polymer results which may be studied by circular dichroism (CD). Thus, poly(ethylenimine) containing the (-)-2-(thymin-1-yl)-propionyl group as the grafted pendant showed exciton coupling of the B_{2u} transition of the base chromophores in the CD, as observed in polynucleotides. This implies at least a local helical order in the stacking. The biological activity of such structures is briefly discussed.

INTRODUCTION

Since the late 1950's, a myriad of synthetic polynucleotide models have been made (SHOMSHTEIN & GILLER, 1976; JONES, 1979; TAKEMOTO and INAKI, 1981); a number have been studied in aqueous solution. Notable among backbone-modified systems is the elegant work on poly-(9-vinyl adenine) and poly(1-vinyl uracil) (KAYE and CHOU, 1973, 1975; PITHA, 1977). These polymers interact with natural polynucleotides; the former inhibits the replication of Friend leukemia virus in mice. In order to study such analogs in aqueous environments and to maximize the potential for biological activity (LUDWICK and OVERBERGER, 1980), we have designed hydrophilic systems of high structural order. Again in analogy with natural polynucleotides, we have placed a chiral center adjacent to the chromophore in our systems. This permits study by CD in addition to the usual UV hypochromic effects. Three linear polymer backbones have been used: poly(ethylenimine)(PEI), poly(vinylamine)(PVam), and poly(dehydroalanine)(PDA). In the first two cases,

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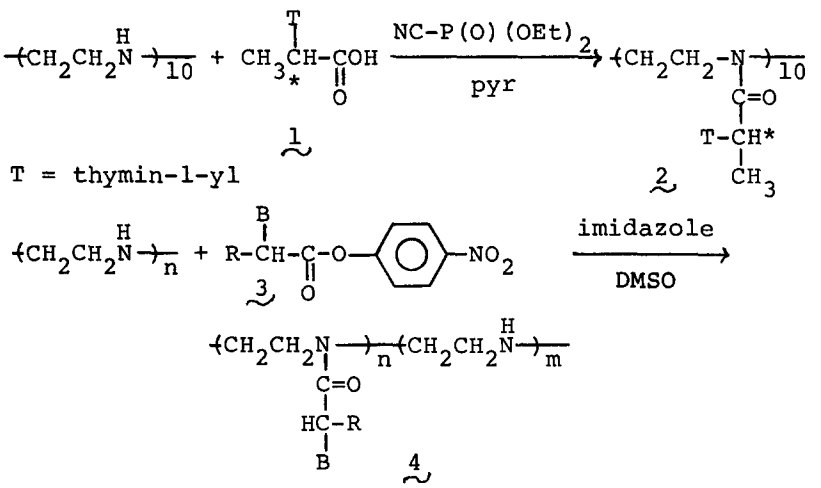
polymer grafting reactions were used to incorporate nucleic acid base moieties; in the latter case, free-radical polymerization of vinyl monomers gave high molecular weight analogs.

RESULTS AND DISCUSSION

Poly(ethylenimine) Analogs

Linear PEI's of various molecular weights have been used for grafting nucleic acid base derivatives as summarized in Scheme 1. The PEI's were obtained either synthetically (SAEGUSA et al., 1972) or by acidic hydrolysis of linear poly(N-propanoylethylenimine) from Dow Chemical Co.

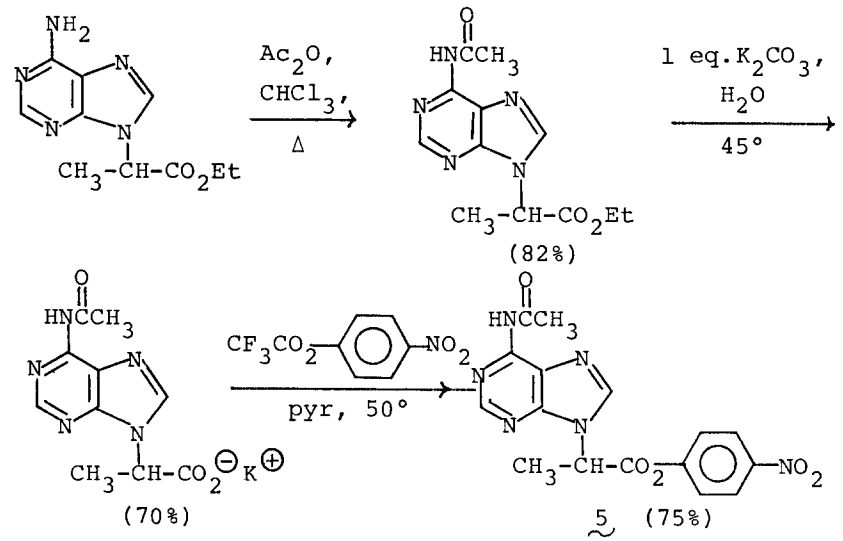
Scheme 1. Nucleic Acid Analogs Based on PEI



#	R	B	Composition
4a	CH_3	adenin-9-yl	n+m=10, n=5.7
4b	CH_3	thymin-1-yl	n=10, m=0
4c	CH_3	6-acetyl adenin-9-yl	n+m=30, n>27
4d	CH_3	6-acetyl adenin-9-yl	n+m=100, n>90
4e	CH_3	thymin-1-yl	n=30 or 100, m=0
4f	CH_3	uracil-1-yl	n=30 or 100, m=0
4g	$(CH_3)_2CH$	thymin-1-yl	n=30 or 100, m=0

Grafting was carried out using either a direct approach with diethyl phosphocyanidate in the case of optically active 1 or via racemic p-nitrophenyl active esters 3, giving analogs 2 and 4. These polymers indicate similarity to natural nucleic acids; significant hypochromicity vs. monomer models was observed for all of the compounds in aqueous solution (e.g., 45.4% for 4a and 36.8% for 4b) as well as for the dimer models made from sym-dimethylethylenediamine (OVERBERGER and MORISHIMA, 1980a; MORISHIMA and OVERBERGER, 1979).

Although 2 or 4b and 4a showed complementary base pair interactions in continuous variation mixing experiments, the low percent graft of 4a make elucidation of the nature of this complex difficult. To improve the percent graft, protection of the 6-NH_2 group of adenine was carried out by the following route:



Compound 5 could be grafted much more cleanly giving highly water-soluble 4c and 4d. Deprotection is effected in dilute aqueous HCl. Experiments are in progress to study the interactions of these polymers with 4e. When the pendant is optically active as in 2, the CD spectrum revealed splitting of the B_{2u} transition by exciton interaction of the stacked bases, implying a helical order (OVERBERGER and MORISHIMA, 1980b). Placing an L-proline spacer group between the backbone and the propionyl moiety permitted stacking, but destroyed any screw sense (OVERBERGER and MORISHIMA, 1980c).

Uracilyl polymers 4f and polymers 4g, derivatives of 3-methyl butyric acid, have been synthesized and examined spectroscopically (LUDWICK and OVERBERGER).

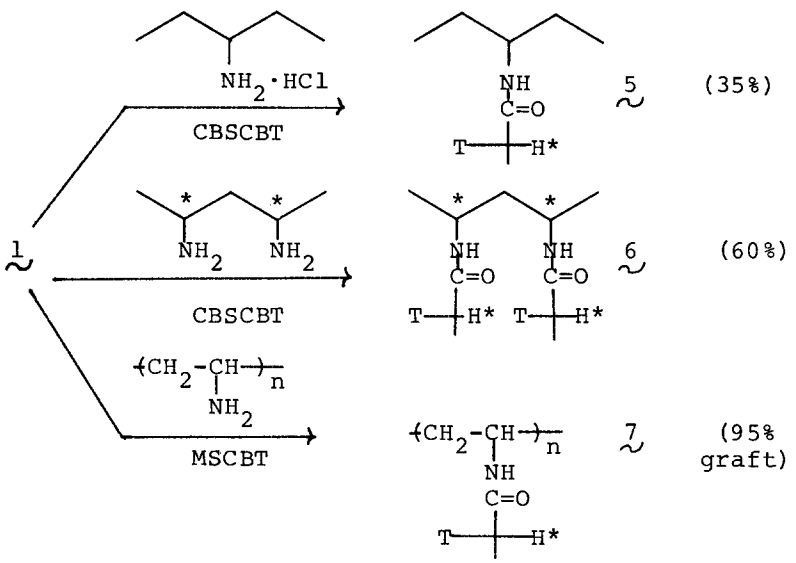
The bulky R group of the latter favors trans amide bonds but may also sterically impede stacking. NMR shows similar conformational populations, whether R = CH_3 or $(CH_3)_2CH$, and hypochromicities are comparable. Hypochromicities decrease as pH increases due to electrostatic repulsion between the charged rings, but increasing the ionic strength even at high pH's increased the hypochromicity. Studies in DMSO/H_2O solutions showed increased base-stacking in the more aqueous solvents; for polymer 4f with n = 100, a marked increase was noted. Since significant activity against influenza A/Victoria/75 was observed in 6% aqueous DMSO solution for 4f when n = 100 (but not when n = 30), water may play some role in the ordering of 4f and similar polymers. Polymer 4f and its monomer model also interact with poly A in TRIS buffer solutions. Detailed biological testing of 4 and the synthesis of higher molecular weight grafts will be carried out; synthesis of optically active 4 will permit further CD studies. Similar chemistry is proving useful for analogous guanine, cytosine, and carbazole derivatives.

Poly(vinylamine) Analogs

The grafting of compounds 3 to linear PVam of molecular weight 6,000 to 150,000 (prepared either by hydrolysis of poly(vinyl acetamide) or poly(vinyl-t-butyl carbamate) has proven to be more difficult than in the case of PEI. This may be due to the low solubility of PVam, which could be dissolved in DMF or DMSO only by adding an acidic compound such as 1-hydroxybenzotriazole. Under these homogeneous conditions, many peptide-coupling reagents gave high percent grafts, but caused severe racemization. Several sulfonic acid esters of hydroxybenzotriazoles (ITOH et al., 1978) however have proven effective in reactions of 1 to give monomer, dimer, and polymer models. These reagents gave good yields, high percent grafts, and little racemization in DMF with pyridine as base (Scheme 2). Treatment of 2,4-pentanedione with hydroxylamine gave the dioxime which was reduced and acidified yielding the dihydrochloride salt of 2,4-diaminopentane. Reaction of the separated meso, (+) and (-) stereoisomers of 2,4-diaminopentane (APPLETON and HALL, 1970) gave diastereomers 6 which are being separated by HPLC to give dimer models for syndiotactic, heterotactic, and isotactic 7. Detailed spectroscopic studies on the effects of tacticity on the solution behavior of these systems are in progress.

Poly(dehydroalanine) Analogs

Although many polyanionic nucleic acid analogs have been made by copolymerization (HOFFMANN, 1979; MAGGIORA et al., 1977), few homopolymers of this type



T = thymin-1-yl
MSCBT = methanesulfonyl-4-chlorobenzotriazole
CBSCBT = 4-chlorobenzenesulfonyl-4-chloro-benzotriazole

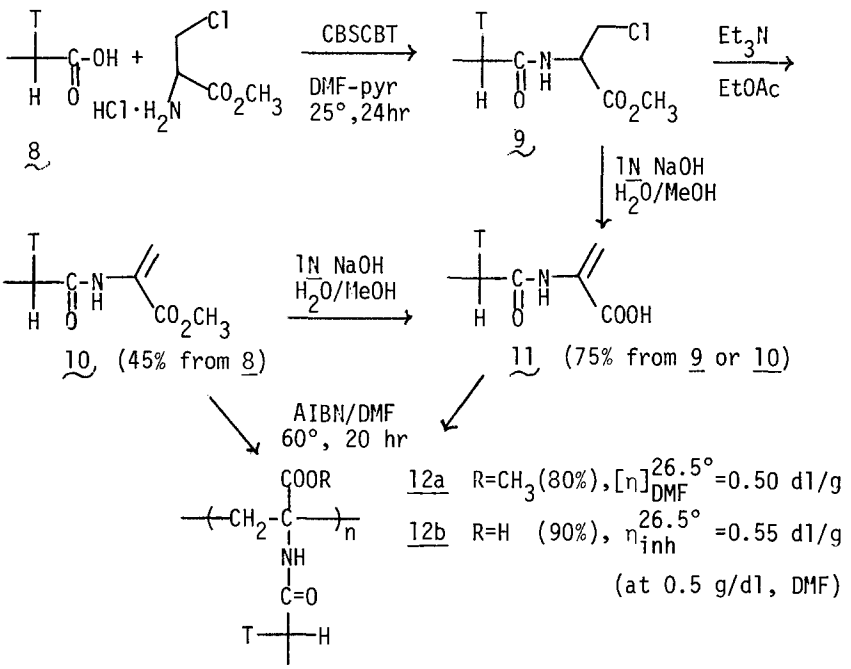
Scheme 2. Nucleic Acid Analogs Based on PVam.

have been reported. A homopolymer would have greater structural regularity, characteristic of polynucleotides, the negative charge should increase water solubility and might be important with respect to biological activity.

Recent work in the literature on water-soluble poly(N-acetyl dehydroalanine) (ASQUITH et al., 1978) led us to investigate this backbone. After a report on the use of β-chloroalanine methyl ester as a synthon for a dehydroalanine monomer appeared (MATHIAS, 1980), we found a modification applicable to our system, as shown in Scheme 3. The coupling conditions used in the PVam series formed the new amide bond; after removal of by-products, dehydrochlorination with Et_3N in EtoAc proceeded smoothly affording monomer 10, which could be hydrolyzed in base (PHOTAKI, 1963) to give the dehydroalanine monomer 11.

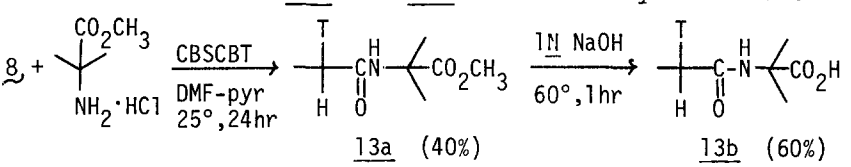
Both 10 and 11 polymerize readily to high molecular weight products 12 as indicated by spectral and viscosity data. Polymer 12b showed marked polyelectrolyte behavior in DMF, with a large increase in viscosity at high dilution. Solubility of the polymers is excellent: both are soluble in DMF and DMSO; 12a is soluble in TFE and $CHCl_3$/MeOH, while, most impor-

Scheme 3. Nucleic Acid Analogs Based on PDA



tantly, 12b is very soluble in neutral water.

Monomer models 13a and 13b were also synthesized:



Significant UV hypochromicity is observed for 12 vs. 13 in aqueous solutions. The results in DMSO/H_2O are shown in Figure 1. The behavior of 12a is in accord with the results obtained for 4f and 4g, while 12b shows the opposite trend. If 12b is assumed to be mainly syndiotactic with trans amide bonds, then the maximum separation of ionized carboxyl groups expected in organic solvents would give a high degree of base-stacking.

Further insight into the secondary structure of 12 should be provided by CD. Preliminary experiments have shown that by using optically active 8 ($[\alpha]_D^{25°} = -49°$ [TFE, C = 1]), optically active 10 may be obtained and polymerized, yielding 12a with $[\alpha]_D^{25°} = -129°$ (C = 1, TFE). This polymer and polymer 2 exhibit similar CD spectra in TFE. Further work on these analogs and on

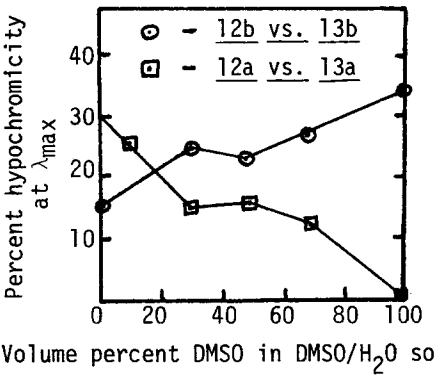


Figure 1. Hypochromicity of 12 versus 13 in DMSO/H_2O solutions.

the synthesis of dehydroalanine monomers containing other nitrogen heterocycles is in progress.

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