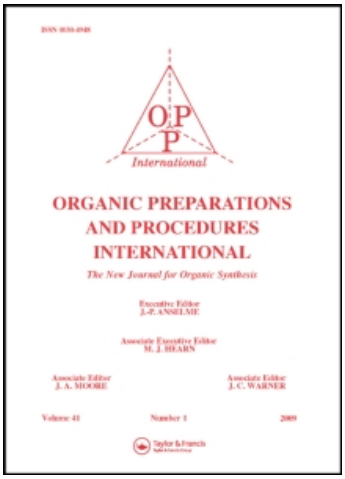

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Ming-Jye Lan[a]; C. G. Overberger[a]
[a] Department of Chemistry and the Macromolecular Research Center, University of Michigan, Ann Arbor, Michigan

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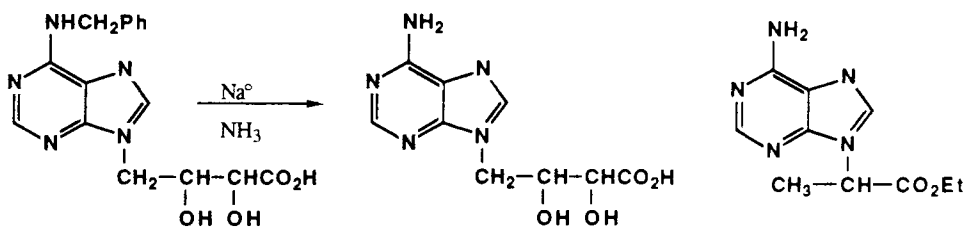
APPROACH TO THE SYNTHESIS OF AN OPTICALLY ACTIVE POLYNUCLEOTIDE ANALOG

Ming-Jye Lan† and C. G. Overberger*

Department of Chemistry and the Macromolecular Research Center
University of Michigan, Ann Arbor, Michigan 48109

Polynucleotides contain a chiral center immediately adjacent to the nucleic acid base, which may give the polymer a stereoregular structure. This stereoregularity has allowed polynucleotides to be studied using circular dichroism.[1] The goal of this work was to synthesize optically active 6 as a model compound which could then be grafted onto poly(ethylenimine) (PEI).



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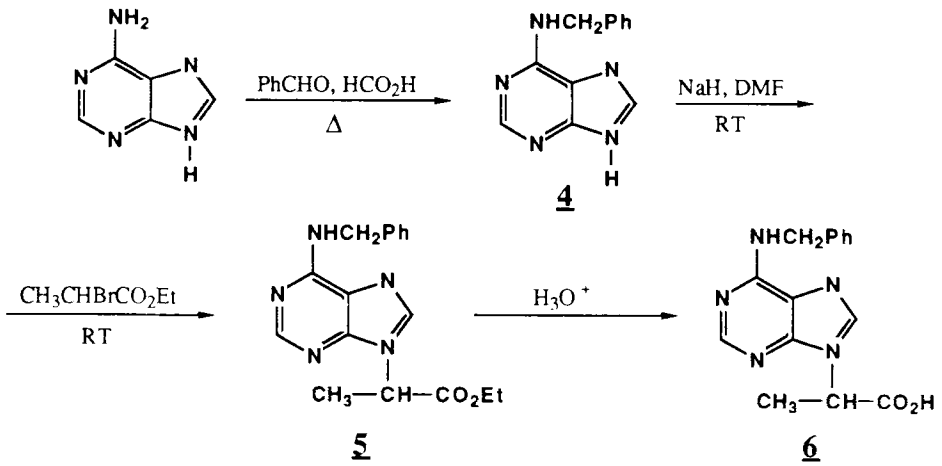
Although Okamura et al.[2] had reported that N^6-benzyleritadenine (1) resisted hydrogenolysis, reductive debenzylation in liquid ammonia produced 2, albeit in poor yield (14%). After several unsuccessful attempts to introduce the benzyl group on the 6-amino group of 3, we found that simple reductive benzylation of adenine[3,4] with benzaldehyde in refluxing formic acid[3] gave N^6-benzyladenine (4) in 49% yield; attempts to increase the yield were unsuccessful. N^6-Benzyladenine was then treated

with sodium hydride at room temperature in DMF then with ethyl 2-bromopropanoate to provide compound 5 in good yield. Analysis of the purified product by TLC (silica gel, 20:1 chloroform-methanol) revealed the presence of only one product with a sharp melting point. The ethyl 2-propanoyl group was assigned to the 9-position of adenine by comparison of



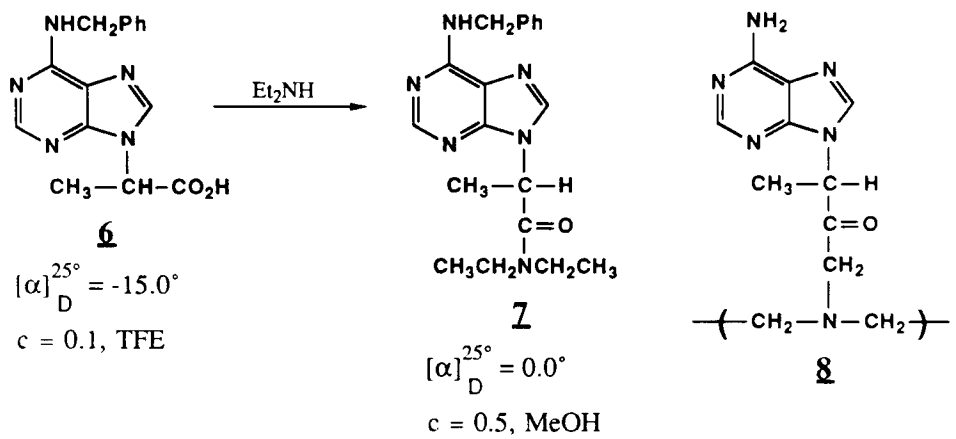
its ultraviolet absorption spectrum (λ_{max} 266 nm, 0.1 N HCl) with that of 9-methyl-N^6-methyladenine (λ_{max} 265 nm, 0.1 N HCl), prepared by an unambiguous route.[5] 2-(N^6-Benzyladenin-9-yl)propanoic acid (6) was then obtained by acid hydrolysis of ester (5), followed by precipitation at its isoelectric pH.

The classical method of resolving acids involving the formation and separation of their diastereomeric salts with an optically active base[6] and carried out with L-strychnine, L-cinchonidine, brucine, quinine and D-(-)-threo-2-amino-1-(p-nitrophenyl)-1,3-propanediol in different solvents, showed that the best results were obtained with L-cinchonidine in 15:1 (v/v) ethyl acetate-ethanol. Optical resolution of 6 was therefore performed on preparative scale. The less soluble diastereomeric salt was purified by seven fractional crystallizations; at least four fractional crystallizations were necessary to achieve a constant value of optical rotation. Evaporation of the combined filtrates saved from fractional

crystallizations provided another diastereomeric salt which was then dissolved in ethyl acetate. The ethyl acetate solution was seeded with the less soluble diastereomeric salt and cooled to -20°. The resulting precipitate was removed to give a purer diastereomer. Passage of the less soluble diastereomer in methanol through IRC-50 ($-CO_2H$) ion exchanger in the acid form, liberated (-)-2-(N^6-Benzyladenin-9-yl)propanoic acid in the solution while L-cinchonidine was retained on the resin. (+)-2-(N^6-Benzyladenin-9-yl)propanoic acid was then isolated from the other diastereomer in the same manner. The yields of both enantiomers were relatively low as a consequence of absorption of the enantiomer on the resin. The use of resins to decompose diastereomeric salts does not usually cause racemization; however, the poor solubility of the enantiomers in non-polar solvents, precluded determination of the enantiomeric purity by use of chiral NMR shift reagents.[7] The reaction of 6 with diethylamine was investigated in order to evaluate its reaction with PEI.

Three different conditions were tried, but only the completely racemized product 7 was isolated (Scheme 1). This result is possibly due

SCHEME 1



to the acidity of the proton at the chiral center, because Chen and Overberger[8] have successfully prepared the optically active polymer 8 using the same DCC/HONB method at 0° described here in the Experimental

Section. In polymer 8 however, the asymmetric center is shielded from the electron-withdrawing effect of the carbonyl group by a methylene carbon (Scheme 1). Grafting of the racemic 2-(N^6-benzyladenin-9-yl)propanoic acid onto linear poly(ethylenimine) will be reported in subsequent articles.

EXPERIMENTAL SECTION

Reagent grade chemicals were used without further purification unless otherwise noted. N,N-dimethylformamide (DMF) was distilled from barium oxide and stored over molecular sieves (4Å). Silica gel thin-layer chromatography (TLC) was performed on Eastman Chromagram sheets with UV visualization at 254 nm. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. All proton NMR spectra were obtained on a Bruker WM-360 (360 MHz) spectrometer; tetramethylsilane (TMS) was used as an internal standard and chemical shifts are expressed in δ values.

N^6-Benzyladenine (6-Benzylaminopurine) (4).- This compound was prepared by the method of Bambury and Siewierski[3] with the following modifications. A mixture of 30.0 g (0.222 mol) of adenine, 27.1 mL (0.266 mol) of benzaldehyde, and 55.5 mL of 98-100% formic acid in a 500 mL round bottom flask was stirred vigorously at reflux for one week; during this time, the reaction mixture turned brown. The formic acid was evaporated under reduced pressure and the resulting pale brown solid was triturated with 400 mL of diethyl ether. The solid was collected and stirred with 800 mL of boiling water for 2 hrs. The insoluble N^6-benzyladenine was collected from the hot mixture and purified by repeated recrystallizations from absolute ethanol using decolorizing carbon to yield 25.0 g (50%) of colorless crystals, mp. 229-230.5°, lit.[3] mp. 229-231.5°.

Ethyl 2-(N^6-Benzyladenin-9-yl) propanoate (5).- Into a 1 L 3-necked round bottom flask fitted with a nitrogen inlet, mechanical stirrer and addition funnel charged with ethyl 2-bromopropanoate (28.8 mL, 0.222 mol) were added 25.0 g (0.110 mol) of N^6-benzyladenine, 4.90 g (0.122 mol) of a hexane-washed 60% sodium hydride-mineral oil dispersion, and 500 mL of dry DMF. The mixture was stirred at room temperature for 2 hrs resulting in a

white suspension of sodium adenine. Ethyl 2-bromopropanoate was then added dropwise over 3 hrs at room temperature under vigorous stirring; stirring was continued for 4 hrs. The solvent was removed from the homogeneous yellow solution by rotary evaporation under high vacuum. The resulting viscous oil was stirred with 500 mL of chloroform overnight and insoluble sodium bromide was removed by filtration. Solvent evaporation gave a viscous oil which was crystallized by the addition of ether and cooling to -20°. Two recrystallizations from acetone (cooled to -20°) gave 28.6 g (80%) of pure ethyl 2-(N^6-benzyladenin-9-yl)propanoate as white crystals, mp. 97-99°. Analysis of this compound by TLC (silica gel) in 20:1 chloroform-methanol showed one spot at R_f = 0.54.

NMR (DMSO-d_6): δ 8.41 (1H, br s, Ad-N<u>H</u>Bn), 8.29, 8.20 (2H, 2 s, Ad$C_{2,8}$-<u>H</u>), 7.29 (5H, m, CH_2C_6<u>H_5</u>), 5.42 (1H, q, CH_3C<u>H</u>), 4.72 (2H, br s, C<u>H_2</u>C_6H_5), 4.14 (2H, q, C<u>H_2</u>CH_3), 1.81 (3H, d, C<u>H_3</u>CH), 1.15 (3H, t, CH_2C<u>H_3</u>).

<u>Anal</u>. Calcd for $C_{17}H_{19}N_5O_2$: C, 62.78; H, 5.84; N, 21.53

Found: C, 62.70; H, 5.85; N, 21.40

<u>2-(N^6-Benzyladenin-9-yl)propanoic Acid (6)</u>.- A solution of ethyl 2-(N^6-benzyladenin-9-yl)propanoate (21.0 g, 0.065 mol) in 400 mL of 5<u>N</u> hydrochloric acid was refluxed for 3 hrs and evaporated to dryness. The solid was dissolved in 500 mL of water and the pH of the solution was adjusted to 5.0 with aqueous saturated sodium carbonate to give a white precipitate. The crude product was isolated by suction filtration and recrystallized from methanol to give 12.9 g (66%) of 2-(N^6-benzyladenin-9-yl) propanoic acid as white crystals, mp. 241-244° (dec.).

NMR (DMSO-d_6): δ 8.38 (1H, br s, N<u>H</u>Bn), 8.26, 8.18 (2H, 2 s, Ad$C_{2,8}$-<u>H</u>), 7.26 (5H, m, CH_2C_6<u>H_5</u>), 5.30 (1H, q, CH_3C<u>H</u>), 4.70 (2H, br s, C<u>H_2</u>C_6H_5), 1.79 (3H, d, C<u>H_3</u>CH).

<u>Anal</u>. Calcd for $C_{15}H_{15}N_5O_2$: C, 60.62; H, 5.05; N, 23.56

Found: C, 60.78; H, 5.29; N, 23.54

Resolution of (+)-2-(N^6-Benzyladenin-9-yl)propanoic Acid (6). Preliminary Test on Resolution.- Preliminary experiments were carried out with common alkaloids and some synthetic bases in different solvents. Compound 6 (0.001 mol) and the base (0.001 mol) were dissolved in small amounts of hot solvent. Crystals which formed upon cooling in the refrigerator overnight were removed by filtration, and the acid was liberated using IR-120 CP ion exchange resin (acid form) and purified by recrystallization from methanol. The optical rotation of the partially resolved acid was measured in 2,2,2-trifluoroethanol (TFE). The best results were obtained with L-cinchonidine in a 15:1 ethyl acetate-ethanol mixture which yielded $[\alpha]_D^{25}$ = -4.0° (c = 0.5, TFE). Thus, resolution of the acid was performed with L-cinchonidine in ethyl acetate-alcohol (15:1) as follows. A solution of 5.00 g (17 mmol) of the racemic acid and 4.95 g (17 mmol) of L-cinchonidine in 200 mL of methanol was refluxed for 1 hr. Complete removal of the solvent under reduced pressure gave a white foam, to which was added under reflux about 400 mL of a 15:1 ethyl acetate-ethanol solution until all the solid dissolved. The salt was allowed to crystallize first at room temperature and then in the refrigerator overnight. The salt was then collected, dried and recrystallized from the mixed solvent. The specific rotation of the salt reached a nearly constant value after four recrystallizations. Seven recrystallizations gave 4.00 g (80% yield of one diastereomer) of crystals with $[\alpha]_D^{25}$ = -78.7° (c = 1.0, CH_3OH), mp. 132-138°.

Anal. Calcd for $C_{34}H_{37}N_7O_3$: C, 69.02; H, 6.30; N, 16.57

Found: C, 68.84; H, 6.53; N, 16.57

This diastereomeric salt (3.50 g, 6.0 mmol) was dissolved in 50 mL of methanol and eluted slowly through a 2.0 cm X 6.0 cm column packed with IRC-50 ion exchange resin (acid form). When the compound had eluted completely from the column as established by UV detection, the eluent was

concentrated to dryness under reduced pressure. The resulting white solid was recrystallized twice from a 1:2 ethanol-water mixture to give 2.27 g (45% yield of one enantiomer) of (-)-2-(N^6-benzyladenin-9-yl)propanoic acid, mp. 207° (cloudy) and 240-242° (dec.), $[\alpha]_D^{25}$ = -15° (c = 0.1, TFE). Determination of the optical purity using chiral shift reagents was not possible since the compound was insoluble in non-polar solvents. The NMR spectrum was identical to that of 6.

The (+)-enantiomer was isolated as follows. The filtrates from the recrystallizations of the diastereomeric salt were evaporated to dryness under reduced pressure. To the resulting tan foam was added, under reflux, ethyl acetate until all the solid dissolved. The solution was cooled to room temperature, seeded with the previously isolated diastereomer, and stored in a freezer (-20°) for one week. After removal of the precipitated salt (2.10 g) by filtration, the filtrate was evaporated to dryness. A minimum amount (about 50 mL) of methanol was added to dissolve the diastereomeric salt. The method of liberating the (-)-enantiomer was used to produce 1.76 g (35% yield of one enantiomer) of (+)-2-(N^6-benzyladenin-9-yl)propanoic acid, mp. 227° (cloudy) and 240-242° (dec.), $[\alpha]_D^{25}$ = +12° (c = 0.1 TFE). No attempt was made to determine the optical purity of this compound due to its poor solubility in non-polar solvents. Its NMR spectrum was identical to that of 6.

Attempted Synthesis of (-)-N,N-Diethyl 2-(N^6-Benzyladenin-9-yl)propanamide (M-(-)-BnA (7).

(a) To a solution of (-)-2-(N^6-benzyladenin-9-yl)propanoic acid (0.20 g, 0.7 mmol) in 5 mL of dry DMF at 0° was added N-hydroxy-5-norbornene-2,3-dicarboximide (HONB) (0.12 g, 0.7 mmol) followed by N,N'-dicyclohexylcarbodiimide (DCC) (0.14 g, 0.7 mmol) in one portion. The solution was stirred at 0° for 4 hrs, after which time was added 0.08 mL (0.74 mmol) of diethylamine. Stirring was continued at room temperature for 18 hrs; the

mixture was filtered and evaporated, and the residue was dissolved in 5 mL of ethyl acetate, cooled, and filtered again to completely remove dicyclohexylurea. The filtrate was washed successively with water, 5% sodium bicarbonate solution, water, then dried (anhydrous magnesium sulfate) and evaporated to dryness. After two recrystallizations from 1:3 tetrahydrofuran/hexane, white flakes were obtained with $[\alpha]_D^{25} = 0.0°$ (c = 0.5, methanol), mp. 130-132° (identical to the racemic monomer model).[9]

(b) Procedure **a** was used but in this case 1-hydroxybenzotriazole (HOBT) substituted for HONB and the suspension was stirred at 0° for 18 hrs after the addition of diethylamine. White flakes were obtained after workup, mp. 130-132°, $[\alpha]_D^{25} = 0.0°$ (c = 0.5, methanol).

(c) Diethylamine (0.17 mL, 1.7 mmol) in 10 mL of dry pyridine was cooled to -20°. (-)-2-(N^6-Benzyladenin-9-yl)propanoic acid (0.5 g, 1.7 mmol) followed by diethylphosphoryl cyanide (DEPC) (0.71 g, 4.4 mmol) were added in one portion. After stirring at -20° for 24 hrs, a second portion of DEPC (0.11 g, 0.7 mmol) was added to the resulting brown solution and stirring continued for an additional 24 hrs. Pyridine was then removed by rotary evaporation under high vacuum. The resulting viscous oil was dissolved in 25 mL of chloroform and washed successively with water, 5% sodium bicarbonate solution, water, then dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated to an oil which was recrystallized twice from ether to give white flakes, mp. 129-131°, $[\alpha]_D^{25}$ = 0.0° (c = 0.1, TFE).

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† Present address: Allied-Signal, Inc. Corporate Technology, P. O. Box 1021R, Morristown, NJ 07960.

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