a water bath. Catecholborane (2.6 mmol, a 5% excess) was added dropwise as a neat liquid to the stirred reaction mixture.

Aliquots were withdrawn at various time intervals, quenched with water, and analyzed by GLC or NMR.

Isolation. Reduction of aldehydes, ketones, acids, acid chlorides, anhydrides, and esters produces the corresponding alkoxy-1,3,2-benzodioxaboroles. The reduction of palmitic anhydride is representative of the general procedure utilized in this study. Palmitic anhydride (2.15 g, 4.32 mmol) was placed in a 25-ml flask which was assembled as described previously. Chloroform (70 ml) was then added and catecholborane (2.03 ml, 18.6 mmol) was added dropwise. The mixture was refluxed until the reduction was complete (3 days, monitored by NMR). The solution was extracted with one 25-ml portion of H_2O followed by six 25-ml extractions using a 1.0 N NaOH solution to remove catechol. The solution was then dried and separated using column chromatography; the column support was silica gel (Sargent-Welch, 60–200 mesch). The hexadecanol was eluted using a ligroin–ether mixture (98 and 2%, respectively). The first material eluted from the column was the hexadecanol (96% isolated, 2.02 g, 8.24 mmol).

Characterization of Products. The spectral data and physical constants of a number of products obtained via selective reduction with CB are summarized in Table VII.

Registry No.—Borane, 13283-31-3; catechol, 154-23-4; CB, 274-07-4.

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Simple Models of Nucleic Acid Interactions. 1. Base–Base Interactions in 1,2-Di(adenosin-N^6-yl)ethane and 1,4-Di(adenosin-N^6-yl)butane[1a,b]

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Treatment of 6-chloro-9-β-D-ribofuranosylpurine (I) with 1,2-diaminoethane in dimethylformamide at room temperature in the presence of triethylamine gave 1,2-di(adenosin-N^6-yl)ethane (IIIb). Compound IIIb was also prepared by coupling of I with N^6-(2-aminoethyl)adenosine (IVa). Similarly, condensation of 6-chloro-9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)purine (V) with IVa afforded 2′,3′-*O*-isopropylidene-1,2-di(adenosin-N^6-yl)ethane (VI), a derivative of IIIb with functionally differentiated ribose residues. Coupling of I with 1,4-diaminobutane gave 1,4-di(adenosin-N^6-yl)butane (IIIc) and N^6-(4-aminobutyl)adenosine (IVb). UV and CD spectra of IIIb and IIIc in water are consistent with an intramolecular base–base interaction (stacking). Thus, the hypochromism of IIIb is greater than that of IIIc. Both IIIb and IIIc exhibit an increased molecular ellipticity in CD spectra over the corresponding model compounds VIIa and VIIb. This increase is more pronounced in IIIc than IIIb. In 0.01 N HCl IIIb still exhibits a considerable hypochromism whereas that of IIIc virtually disappeared. By contrast, the CD spectra of IIIb and IIIc show a sharp drop in the molecular ellipticity which in both cases does not substantially differ from that in model compounds VIIa or VIIb. The effect of protonation on stacking, UV and CD spectra of IIIb and IIIc is discussed.

Interactions between the strands of nucleic acids are essential for the biological roles of both DNA and RNA in phenomena such as replication of DNA, transcription of genetic information from DNA to RNA, codon–anticodon interaction of mRNA with tRNA, etc., wherein two molecules (strands) approach one another closely enough to form a complex. The stability of these complexes derives mainly from the formation of specific hydrogen bonds between complementary bases (Watson–Crick or "wobble" pairing). In another type of interaction, the portions of nucleic acid molecules do not form hydrogen-bonded structures but, nevertheless, interact with each other through base stacking: the heterocyclic residues in the DNA or RNA are held in parallel planes in a sandwichlike arrangement. This base stacking is of importance for maintaining the proper secondary structure of DNA and RNA. It may also be of significance in some other cases where portions of DNA or RNA molecules are close enough but cannot form a hydrogen-bonded complex because of lack of the corresponding complementary bases. This situation may arise in the crucial step of protein synthesis where the pepti-

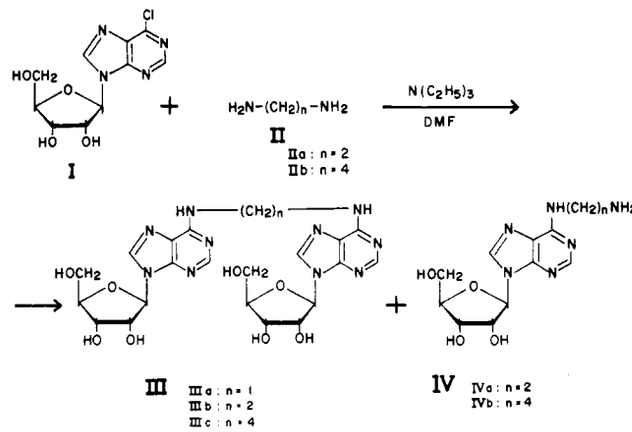
dyl- and aminoacyl-tRNA may interact during formation of the new peptide bond.[2] Because both molecules contain the identical 3′ terminal sequence (C-C-A), it would be difficult to envision a Watson–Crick type of interaction. However, as can be shown with Corey–Pauling–Koltun (CPK) space-filling models, base stacking of both C-C-A ends would be possible bringing together peptidyl and aminoacyl moieties in precise stereoelectronic fashion necessary for the synthesis of the peptide bond.[3] Further examination of CPK models has shown that more constrained model systems, such as 1,2-di(adenosin-N^6-yl)ethane (IIIb) and 1,4-di(adenosin-N^6-yl)butane (IIIc), would adequately approximate the situation. Thus, the terminal adenosine units of peptidyl- and aminoacyl-tRNA may be represented by one molecule containing peptidyl and aminoacyl moiety on the 3′ hydroxyl groups. More recently, two adenosine units have been covalently joined through their respective ribose moieties to form another type of a constrained model of a transition state of protein biosynthesis.[4] The model, as previously indicated, inhibited protein biosynthesis in a ribosomal system.[4] However, no data on possible stacking of adenine rings are available. In addition, it has been reported that the phosphate derivative of IIIa inhibits the mitotic activity of the cells and reproduction of certain viruses.[5] An interesting antitumor activity of 1,2-di(adenin-N^6-yl)ethane has also been briefly described.[6] More recently, similar derivatives (IIIa) have been considered as potentially valuable metabolites for use in cancer therapy.[7] All these considerations led us to the synthesis and spectroscopic investigation of compounds IIIb and IIIc.

The syntheses of 1,2-di(adenin-N^6-yl)ethane[6a,b,8] and 1,3-di(adenin-N^6-yl)propane,[9] as well as 1,1-di(adenosin-N^6-yl)methane (IIIa),[10] and compound IIIb[11] have been described. α,ω-Di(adeninyl)alkanes and some analogous compounds derived from other nucleic acid bases have been a subject of spectroscopic studies.[9,12] The former are regarded as good models of base–base interactions in nucleic acids because of absence of complicating factors (hydrogen bonding of ribose residues and electrostatic interactions of phosphodiester linkages). However, no data on a possible base–base interaction (stacking) in 1,1-di(adenosin-N^6-yl)methane (IIIa) and the corresponding homologues IIIb and IIIc are available to date. Compounds IIIb and IIIc are more complicated since both adenines carry a ribose residue, although the phosphodiester linkage is missing, and thus represent a link in stacking models between simple bases[9,12] and dinucleoside phosphates whose base–base interactions have been extensively studied.[13] The presence of the D-ribofuranose moiety makes study of IIIb and IIIc by a simple CD technique possible. The comparison of results obtained from UV and CD spectra is also of interest.

Results and Discussion

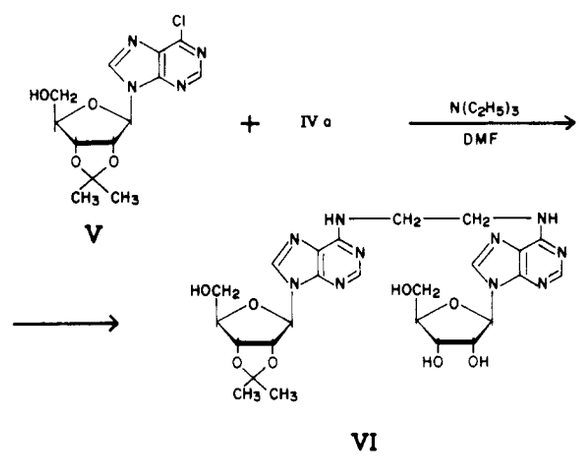
Synthesis. The preparation of the title compounds (IIIb and IIIc) followed a general route which was worked out some time ago[14] for the synthesis of various N^6-substituted adenosines from the 6-chloro-(9-β-D-ribofuranosyl)purine (I) and corresponding amine in the presence of triethylamine in dimethylformamide (DMF). The method has a distinct advantage of working at room temperature. Thus, the reaction of I with 1,2-diaminoethane (IIa) in stoichiometric amounts in the presence of triethylamine in DMF gave IIIb in 30% yield in addition to recovered starting material I (26%) along with N^6-(2-aminoethyl)adenosine (IVa, 21%, Scheme I). Compound IVa can also be employed as a convenient starting material for the preparation of IIIb. The synthesis of IIIb was described,[11] but the yield of the product was rather low (10%). Moreover, the intermediate IVa in the synthesis of IIIb could not be obtained in a crystalline form but as a syrup characterized only by acid hydrolysis to N^6-(2-aminoethyl)adenine.[11]

Scheme I



Our method, which employs a simple ion-exchange separation technique, makes possible the isolation of all reaction products and the preparation of IVa as a crystalline solid. The method of choice for the preparation of IIIb (40% yield) is the reaction of IVa with I (100% molar excess) in DMF using triethylamine as a coupling reagent. The latter procedure made possible the preparation of compound VI with functionally differentiated ribose moieties. Thus, the isopropylidene derivative V was coupled with IVa using triethylamine in DMF to give VI (60%, Scheme II). Product VI may be useful for the preparation of

Scheme II



compounds containing different substituents in the ribose portion. The reaction of I with 5 molar excess of IIb gave predominantly derivative IVb (78%) along with IIIc (21%). The condensation of I (ca. 2 molar excess) with IVb gave bridged nucleoside IIIc in 36% yield. A similar method, reaction of I with the appropriate amine in the presence of triethylamine at room temperature, was employed for the preparation of both spectroscopic models VIIa and VIIb.

NMR spectra of IIIb and IIIc, which supported the proposed structures, failed to reveal any information about base–base interactions as a probable consequence of a poor choice of both concentration (ca. 0.1 M) and solvent (CD_3SOCD_3). Thus, it is known that high concentration favors self-association and, moreover, CH_3SOCH_3 causes a considerable destacking of bases in dinucleoside phosphates.[15] It was not possible to measure NMR spectra in water because of a very limited solubility of IIIb and IIIc. The chemical shifts of H_8, H_2, $H_{1'}$, and the coupling constant $J_{1',2'}$ in IIIb and IIIc are virtually identical with those of the corresponding model compounds VIIa and VIIb, though both H_8 and H_2 in IIIb are slightly less shielded relative to VIIa.

UV and CD spectra. UV and CD studies have provided a considerable body of information about the interaction of both

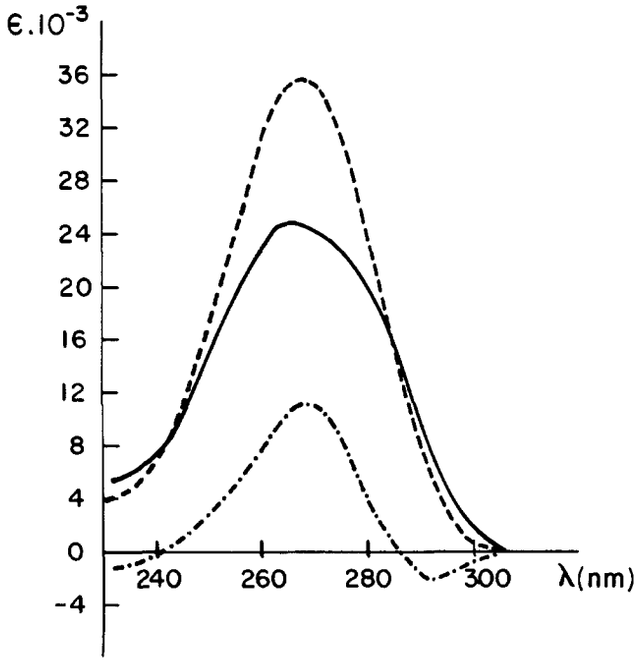


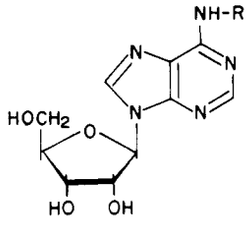
Figure 1. UV spectra of IIIb and VIIa in water (pH 7): —, IIIb: - - -, VIIa; – · – · –, difference spectrum.

Table I. Hypochromism (H) and Hypochromicity (h) of 1,2-Di(adenosin-N^6-yl)ethane (IIIb) and 1,4-Di(adenosin-N^6-yl)butane (IIIc)

Compd	H^a	h^a	H^b	h^b
IIIb	19.2[c]	30.6	14.8	29.3
IIIc	8.2[c]	12.3	None	None

[a] In percent, H_2O (pH 7). [b] In percent, 0.01 N HCl. [c] Values of 14.2 (IIIb) and 6.8 (IIIc) reported in our preliminary report[1b] refer to measurements in distilled water whose pH was not adjusted to 7.

adenine residues in IIIb and IIIc. The measurements were carried out at ca. 50–100 μM, which excluded concomitant intermolecular interactions (self-association) (cf. ref 9). As model compounds we used N^6-ethyladenosine (VIIa) for IIIb and N^6-butyladenosine (VIIb) for IIIc. Similar models (i.e.,



VII

VII a : R = C_2H_5

VII b : R = $CH_3(CH_2)_3$

N^6-propyladenine) have been used in a spectroscopic study of base–base interactions in α,ω-(adeninyl)alkanes.[9,12] Both UV and CD spectra in water are indicative of interaction of adenine residues in IIIb and IIIc. Thus, a hypochromic effect is seen in the UV spectrum of IIIb (Figure 1, Table I) together with a hypsochromic shift of the absorption maximum relative to model compound VIIa and a shoulder at ca. 275 nm.[11] A CD spectrum of IIIb in water[16] exhibited a profound increase in

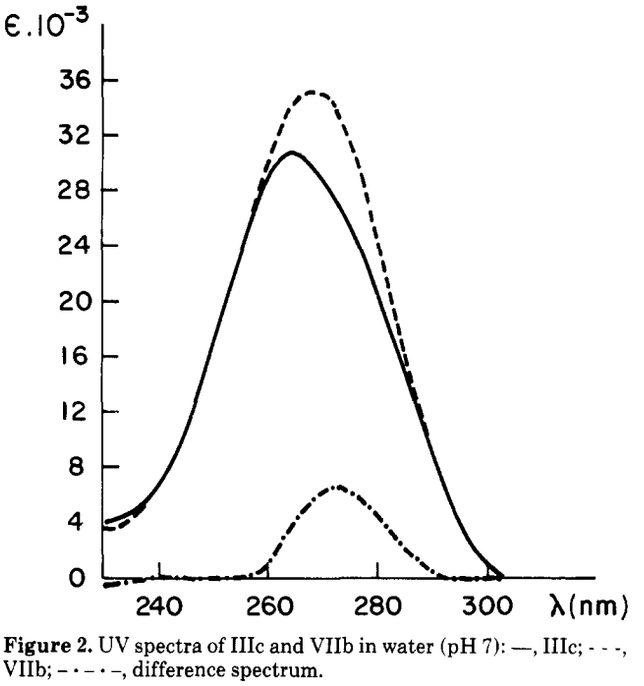


Figure 2. UV spectra of IIIc and VIIb in water (pH 7): —, IIIc; - - -, VIIb; – · – · –, difference spectrum.

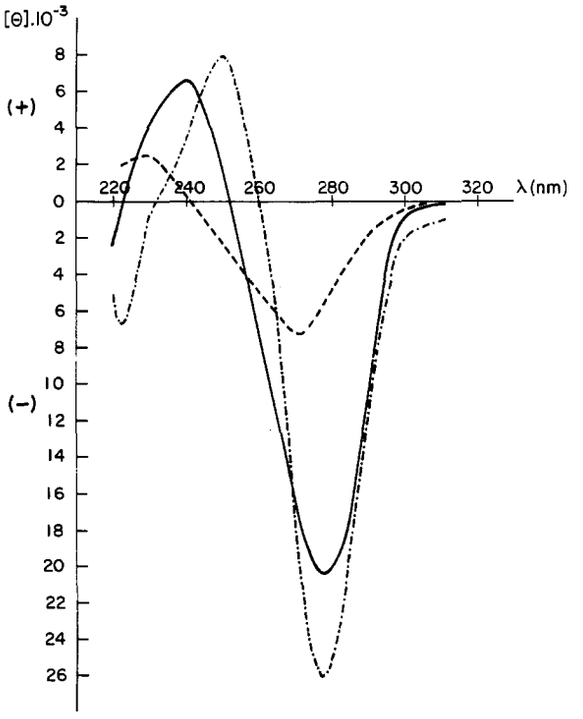


Figure 3. CD spectra of IIIb, IIIc, VIIa, and VIIb in water (pH 7): —, IIIb; – · – · –, IIIc; - - -, VIIa. (The curve of VIIb is almost superimposable on that of VIIa.)

the magnitude of the Cotton effect and a bathochromic shift of its maximum relative to the model VIIa (Figure 3). A similar increase in the intensity of the Cotton effect has been noted earlier for a series of dinucleoside phosphates and explained in terms of intramolecularly stacked structures.[13] Thus, it seems reasonable to assume an extensive base–base interaction in IIIb. The UV spectrum of IIIc in water also showed a hypochromic effect (Figure 2, Table I), although considerably smaller than in IIIb. The pronounced shoulder at ca. 275 nm apparent in IIIb is indistinct with IIIc. The magnitude of the Cotton effect in IIIc is greater than in IIIb but the whole band is narrower (Figure 3). This is surprising because the magni-

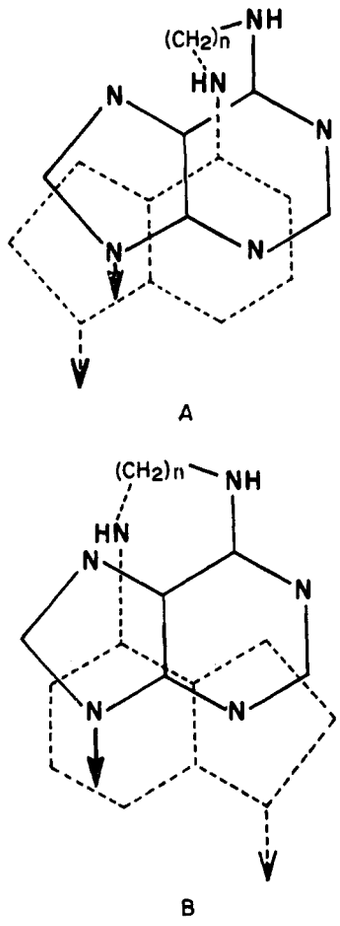


Figure 4. Two stacked conformers of bridged nucleosides IIIb and IIIc (n = 2 or 4). For the sake of simplicity endo nitrogen atoms are omitted from the second purine residues indicated by an interrupted line. Arrow indicates attachment of the ribofuranose. A, imidazole–imidazole and pyrimidine–pyrimidine overlap; B, pyrimidine–imidazole overlap.

tude of the Cotton effect in IIIb and IIIc does not correlate with a sharp drop in hypochromism observed in IIIc relative to IIIb (Table I). It is realized, however, that unlike the UV spectra, the CD can reflect effects other than a simple time-averaged separation of bases such as base orientation and/or conformation in the stack.[17] The latter influences may well be different in IIIb and IIIc. Once again, a bathochromic shift of the maximum of the CD curve is observed relative to VIIb. The differences in hypochromism between IIIb and IIIc undoubtedly reflect the influence of the lengthening of the aliphatic chain and, consequently, a greater time-averaged separation of residues in IIIc. Of interest is also the comparison of hypochromism (H) values of IIIb and IIIc with that of 1,3-di(adenin-N^6-yl)propane. As can be expected, the H value of the latter (15.5)[9] is lower than that of IIIb (19.2). The hypochromism (H) of IIIc (Table I) is very close to that of ApA (6.8)[19] and indeed, as can be seen from CPK models, the distance between stacked adenines in ApA corresponds more to that in IIIc than in IIIb. However, CD spectra clearly indicate important differences between the three diadenosine phosphates[20] and compounds IIIb and IIIc. Thus, CD curves of IIIb and IIIc are quite similar (apart from an increased ellipticity) to those of simple adenosine derivatives (negative Cotton effect), but the adenosine phosphodiesters exhibit one positive and one negative Cotton effect. This again stresses the importance of the phosphodiester group and handedness of its screw axis for CD properties of oligonucleotides.[20]

UV and CD spectra of IIIb and IIIc in acid are also of interest. The stacking of bases in dinucleoside phosphates (ApA) and thus the hypochromic effect is usually greatly reduced in acid because of unfavorable electrostatic effects associated with protonation of bases.[19] It is, therefore, of interest that IIIb shows a considerable hypochromism in 0.01 N HCl while that of IIIc virtually disappeared (Figure 5, Table I). The same trend was observed in CD spectra of IIIb and IIIc, respectively. In both cases, there is a profound decrease in the magnitude of the Cotton effect (ellipticity) in 0.01 N HCl (Figure 6). Once again, the sharp drop in the molar ellipticity does not correlate with a significant hypochromism of IIIb. It is also of interest to note that both IIIb and IIIc exhibit a hypsochromic shift of the UV absorption maximum and a bathochromic shift of the maximum of the Cotton effect relative to the corresponding model compounds VIIa and VIIb (Figures 5 and 6).

Scheme III is proffered in an attempt to rationalize the results. It is reasonable to assume that addition of one proton

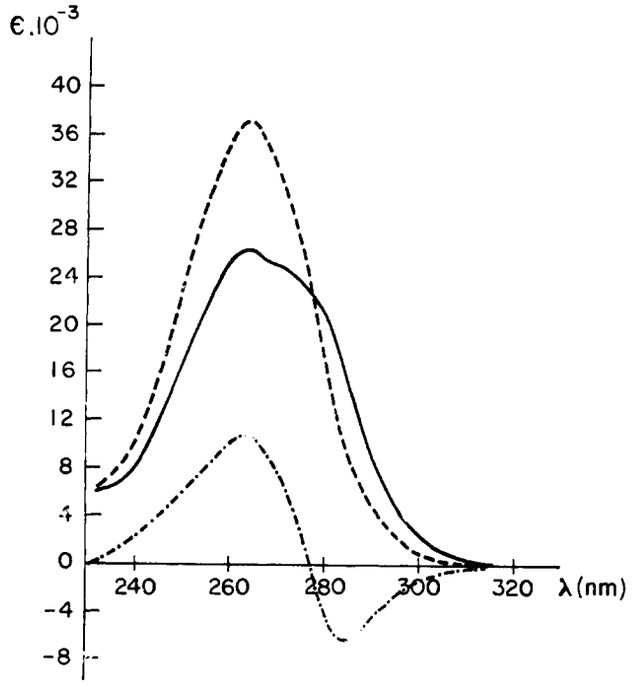


Figure 5. UV spectra of IIIb and VIIa in 0.01 N HCl: —, IIIb; - - -, VIIa; – · – · –, difference spectrum.

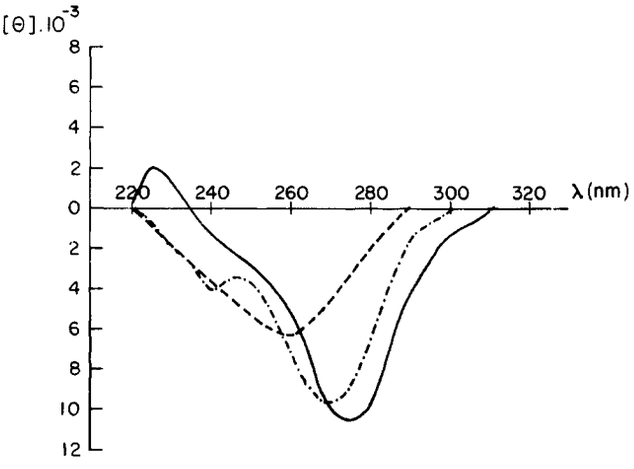
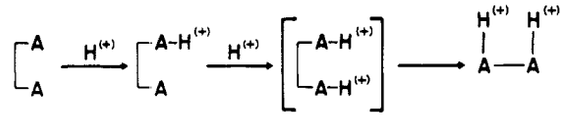


Figure 6. CD spectra of IIIb, IIIc, VIIa, and VIIb in 0.01 N HCl: —, IIIb; – · – · –, IIIc; - - -, VIIa. (The curve of VIIb is almost superimposable on that of VIIa.)

Scheme III



A = adenosin-N^6-yl, $\left[\begin{smallmatrix}A\\A\end{smallmatrix}\right.$ designates a stacked species, A—A stands for an unstacked form

to either IIIb or IIIc need not necessarily lead to destacking of bases. However, the situation may change dramatically with the addition of a second proton because of electrostatic forces which will result in maximum separation of base residues. Therefore, there is little probability of base stacking in the case of a diprotonated species.[19] Furthermore, the degree of protonation may be influenced by the time-averaged separation of bases. Thus, while it is not necessary to expect a substantial difference in the ease of attachment of one proton in both IIIb and IIIc, it seems quite likely that the attachment of the second proton to IIIb (assuming a monoprotonated stacked structure, Scheme III) would be more difficult than to IIIc. In this respect, the situation is considerably different from that found in most dinucleoside phosphates in acid where a negatively charged phosphodiester grouping may compensate one positive charge and thus enable the addition of the second proton. Thus, the UV data may reflect a difference in the base stacking dependent on the extent of protonation of IIIb and IIIc. The UV spectrum of IIIb in 0.01 N HCl (Figure 5, Table I) suggests a considerable stacking due probably to the presence of a monoprotonated form. It is not surprising that differences in protonation may also influence the CD properties. On the other hand, the CD data may reflect, in addition to destacking, changes in orientation or conformation of the base in the protonated stack.

Compounds IIIb, IIIc, IVa, IVb, VIIa, and VIIb were tested in murine leukemia L1210 in vitro system. The first five derivatives did not inhibit DNA synthesis in this system at 0.5–1.0 mM whereas VIIb was inhibitory (ID_{50} 22 μM). It is of interest to note that the latter compound was also reported to inhibit the growth of sarcoma 180 cells, mouse mammary carcinoma TA-3 cells, and murine leukemia L1210 in vivo.[21] The results of the biological testing of the above compounds and some related derivatives in a human cell system will be reported elsewhere.[22]

Experimental Section

General Procedures. Evaporations were carried out with a Büchi rotary evaporator in vacuo at a bath temperature below 35 °C unless stated otherwise. Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. Samples for analysis were dried for 8 h at 10^{-3} mm over P_2O_5 at 100 °C unless stated otherwise. Microanalyses were performed by Micro-Tech Laboratories, Inc., Skokie, Ill. Thin layer chromatography (TLC) was performed on 6 × 2 cm, precoated, silica gel F-254 aluminum foils (Merck, Darmstadt, Germany) in solvents S_1 (dichloromethane–methanol, 9:1) and S_2 (dichloromethane–methanol, 4:1). For preparative TLC 4 mm thick, 35 × 15 cm loose layers of silica gel (70–325 mesh ASTM, Merck, Darmstadt, Germany) containing 1% of fluorescent indicator (Luminous Pigment ZS Super No. 54030, Hoechst Corp., Somerville, N.J.) were used. TLC in solvent S_3 (2-propanol–concentrated ammonium hydroxide–water, 7:1:2) was performed on glass plates (6 × 2 cm) coated with microcrystalline cellulose (Avicel) and the above fluorescent indicator. For preparation of the plates see ref 23. For paper chromatography solvents S_3 and S_4 (1-butanol–acetic acid–water, 4:1:5) on Whatman No. 1 paper were used. Paper electrophoresis was conducted on an electrophoresis flat plate (Savant Instruments, Inc., Hicksville, N.Y.) using 0.05 M $Na_2B_4O_7$ (pH 9.0) and 0.05 M sodium citrate (pH 3.5) as buffers on Whatman No. 1 paper at 40 V/cm for 1 h. For R_f values and electrophoresis mobilities see Table II. UV-absorbing compounds were detected using a Mineralight lamp, ninhydrin-positive substances with 0.1% ninhydrin in ethanol. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. Ion-exchange chromatography was performed with Dowex 50 WX 2, 200–400 mesh resin. NMR spectra were obtained using a Varian A-60A spectrometer; $(CH_3)_4Si$ was used as internal standard with CD_3COCD_3, DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) as external reference with CD_3SOCD_3. DMF was dried with Linde molecular sieves, 4A.

Table II. R_f Values and Electrophoretic Mobilities of Products[a]

Compd	R_f (S_3)	R_f (S_4)	Mobility in Borate[b]	Mobility in Citrate[b]
IIIb	0.49	0.40	1.25	0.44
IIIc	0.64	0.54	1.25	0.50
IVa	0.56	0.25	0	1.9
IVb	0.63	0.30	−0.27	2.0
VI	0.83		0.61	0.02
VIIa	0.87	0.75		
VIIb	0.93	0.88		

[a] For details see general procedures. [b] Relative to adenosine = 1.00.

Starting Materials. 6-Chloro-(9-β-D-ribofuranosyl)purine (I) was either a commercial product (Sigma Chemical Co., St. Louis, Mo.) or it was prepared according to the literature.[14] 1,2-Diaminoethane (IIa) and 1,4-diaminobutane (IIb) were products of Aldrich Chemical Co., Milwaukee, Wis.

Ultraviolet (UV) and Circular Dichroism (CD) Measurements. For measurements either analytical samples were used or compounds whose purity checked by NMR, nitrogen analysis, and paper electrophoresis corresponded to that of analytical samples. Samples for measurements accurately weighed (ca. 7 mg) on Cahn electrobalance RTL (Cahn Division, Ventron Instruments, Paramount, Calif.) were dissolved in water whose pH was adjusted to 7 with NaOH. The corresponding aliquots were diluted immediately before the measurement with water (pH 7) or HCl;[24] approximate final concentrations were 50–100 μM. UV spectra of the solutions were scanned at ambient temperature in a 1-cm cell between 200 and 400 nm using a Cary recording spectrophotometer, Model 11. The resultant curves were replotted as molar extinction coefficients ϵ or, in the case of model compounds VIIa and VIIb, 2ϵ against the wavelength. The difference UV spectra were obtained graphically (Figures 1, 2, and 5). A minimum of two sets of measurements was done in each case. To obtain hypochromism H, the recorded UV spectra were integrated[12] by a computer from the vicinity of the absorption minimum (ca. 230 nm) to the zero absorption at long wavelengths (ca. 310–320 nm) in 2-nm intervals. The values of H were then calculated from the corresponding oscillator strengths,[12] $f = 4.32 \times 10^{-9} \int \epsilon\lambda/\lambda^2 \, d\lambda$ and $H = (1 - f^A/2f^B) \times 100$, where f^A is the oscillator strength of IIIb or IIIc and f^B the oscillator strength of the model compound VIIa or VIIb. Hypochromicity values h were calculated from the expression $h = (1 - \epsilon^A{}_{max}/2\epsilon^B{}_{max}) \times 100$ where ϵ_{max} are the appropriate extinction coefficients at λ_{max}.

CD curves were obtained using the solution made for UV spectra on a JASCO optical rotatory dispersion recorder, Model ORD/UV-5, in a CD modification SS-10 (Sproul Scientific, Boulder Creek, Calif.) between 500 and 200 nm at ambient temperature in a 1-cm cell. The CD data were digitized by hand after a smooth curve had been drawn through the data. The results were plotted as molar ellipticities $[\theta]$ (in the case of VIIa and VIIb $2[\theta]$ against the wavelength, Figures 3 and 6).

6-Chloro-9-β-D-(2,3-*O*-isopropylidene)ribofuranosylpurine (V). This compound, which was described earlier,[25] was prepared by a modified[26] method. The mixture of nucleoside I (0.9 g, 3.14 mmol), acetone (0.45 ml, 4.5 mmol), ethyl orthoformate (1.2 ml, 8 mmol), 3.05 M HCl in DMF (1.44 ml, 4.95 mmol), and DMF (25 ml) was briefly shaken and the resultant clear solution was kept for 22 h at ambient temperature. Sodium bicarbonate (0.84 g, 10 mmol) was added followed by concentrated NH_4OH (0.5 ml), and the solids were filtered off and washed with DMF. The filtrate was evaporated at 0.1 mm and room temperature and the residue was partitioned between water (10 ml) and chloroform (2 × 25 ml). The dried ($MgSO_4$) chloroform extract was evaporated to a syrup. Coevaporation with ethanol afforded a white solid, 1.02 g (100%), which was crystallized from a minimum amount of ethanol to give 6.75 g (73%) of V, mp 154–157 °C (lit.[25]

158–159 °C), on TLC (S_1) homogeneous, UV max (95% ethanol) 264 nm (ε 9300), inflex ca. 250 (6400); NMR (CD_3COCD_3) δ 8.47 (s, 2, H_8 + H_2, resolved into two singlets after addition of D_2O), 6.21 (d, 1, $H_{1'}$, $J_{1',2'}$ = 3 Hz), 5.31 (q, 1, $H_{2'}$), 5.00 (q, 1, $H_{3'}$), 4.33 (m, 2, after addition of D_2O 1 proton, $H_{4'}$ + OH), 3.77 (broad t, sharp doublet after addition of D_2O, 2, $H_{5'}$), 1.55 and 1.33 (s, 6, CH_3 of isopropylidene).

1,2-Di(adenosin-N^6-yl)ethane(IIIb)andN^6-(2-Aminoethyl)-adenosine (IVa). A. From I and IIa. The solution of chloro nucleoside I (1 g, 3.48 mmol), 1,2-diaminoethane (IIa, 0.12 ml, 1.8 mmol), and triethylamine (0.61 ml, 4.38 mmol) in DMF (15 ml) was kept for 2 days at room temperature. Triethylamine hydrochloride was filtered off and the filtrate was evaporated at room temperature and 0.1 mm to afford a residue which was dissolved in water (25 ml) and applied to Dowex 50 column (60 ml, H^+ form). Elution with water (600 ml) and evaporation of the eluate yielded 0.26 g (26%) of the starting material I. Further elution with 5% pyridine (600 ml) afforded product IIIb (0.59 g, 30%) which according to electrophoresis (citrate), TLC (S_2), and $AgNO_3$ test contained a small amount of I. Repeated Dowex 50 (H^+) purification (as above) gave IIIb (0.51 g) still contaminated with I. Pure IIIb was obtained by crystallization of this material from 50% ethanol (150 ml): yield 0.2 g (10%); mp >250 °C dec [lit.[11] 251–252 °C (trihydrate)], uniform on paper chromatography (S_3) and electrophoresis ($Na_2B_4O_7$); $[\alpha]^{25}D$ −39° (*c* 0.5, CH_3SOCH_3); UV max (H_2O) 265 nm (ε 24 800), inflex ca. 278 (21 400); (0.01 N HCl) 263 nm (ε 26 300),[27] inflex ca. 278 (22 700); NMR (CD_3SOCD_3) δ 8.29 (s, 1, H_8), 8.16 (s, 1, H_2), 5.87 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6 Hz), the rest of the ribose protons are overlapped with hydroxy and methylene groups in the region of δ 3.5–4.6. Anal. Calcd for $C_{22}H_{28}N_{10}O_8 \cdot 2H_2O$: C, 44.29; H, 5.41; N, 23.48. Found: C, 44.10; H, 5.26; N, 23.63. After drying at 100 °C immediately before the analysis the compound was analyzed for a monohydrate. Anal. Calcd: C, 45.67; H, 5.23; N, 24.21; H_2O, 3.11. Found: C, 45.66; H, 4.96; N, 24.28; H_2O, 3.40.

Elution of the column with 3% aqueous NH_3 (600 ml) afforded compound IVa (0.23 g, 21%): mp 202–204 °C, after recrystallization from water 203–205 °C; $[\alpha]^{22}D$ −69° (*c* 0.5, CH_3SOCH_3); UV max (95% ethanol) 267 nm (ε 17 800); (0.01 N HCl) 274 nm (ε 17 300); NMR (CD_3SOCD_3) δ 8.33 (s, 1, H_8), 8.18 (s, 1, H_2), 5.89 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6 Hz). Compound IVa gives a positive ninhydrin reaction. Anal. Calcd for $C_{12}H_{18}N_6O_4$: C, 46.44; H, 5.85; N, 27.09. Found: C, 46.33; H, 5.81; N, 27.35.

B. From I and IVa. The solution of chloro nucleoside I (1 g, 3.48 mmol), aminoethyl derivative IVa (0.54 g, 1.74 mmol), and triethylamine (1.61 ml, 7.18 mmol) in DMF (20 ml) was kept for 5 days at room temperature. Evaporation at room temperature and 0.1 mm afforded a solid which was partly dissolved in 50% ethanol and stirred for ca. 30 min with Dowex 50 (H^+). The suspension was then applied to a Dowex 50 (H^+) column (total volume 60 ml) and the elution followed the procedure described in the preceding experiment (50% ethanol was used instead of water). Elution with 50% pyridine afforded product IIIb (0.39 g, 40%) identical (UV, NMR) with the sample described above.

2′,3′-*O*-Isopropylidene-1,2-di(adenosin-N^6-yl)ethane (VI). The solution of isopropylidene derivative V (0.33 g, 1 mmol), aminoethyl nucleoside IVa (0.31 g, 1 mmol), and triethylamine (0.7 ml, 5 mmol) in DMF (5 ml) was kept for 4 days at room temperature. Crystalline triethylamine hydrochloride which separated was filtered off and washed with DMF (2 ml). The filtrate was evaporated at room temperature and 0.1 mm. The residue, after trituration with acetone (5 ml), afforded a white solid (465 mg) which was filtered off and washed with acetone (5 ml), containing according to TLC (S_2), product VI and some IVa (no isopropylidene derivative V was detected). This material was dissolved in 5% pyridine, applied to a column of Dowex 50 (25 ml, pyridinium form) and the column was eluted with the same solvent (250 ml). Evaporation of the eluate gave a glassy solid which was washed with acetone–ether to afford 0.4 g (62%) of VI contaminated, according to TLC (S_2), with a slower moving impurity. The above material was dissolved in methanol, and the solution was applied to a loose layer of silica gel and chromatographed in S_2. The main UV-absorbing band was eluted with dichloromethane–methanol (1:1) and the eluate was evaporated. The resultant amorphous solid was washed with acetone–ether to give 140 mg (22%) of VI, mp 154–155 °C (foaming), homogeneous on TLC (S_2) and electrophoresis ($Na_2B_4O_7$). UV (0.01 N HCl) was essentially identical with that of IIIb. NMR (CD_3SOCD_3 + D_2O) δ 8.40 (s, 2, H_8), 8.30 and 8.28 (2 partially overlapped singlets, H_2), 6.18 (d, 1, $H_{1'}$ of 2′,3′-*O*-isopropylideneribofuranose, $J_{1',2'}$ = 3 Hz), 5.95 (d, 1, $H_{1'}$ of ribofuranose, $J_{1',2'}$ = 6 Hz), 5.40 (q, 1, $H_{2'}$ of 2′,3′-*O*-isopropylideneribofuranose), 5.02 (q, 1, $H_{3'}$ of 2′,3′-*O*-isopropylideneribofuranose), the rest of the ribose protons are at δ 4.67 (t, 1, not well resolved) and between δ 3.6 and 4.3, 1.62 and 1.40 (2 s, 6, CH_3). Anal. Calcd for $C_{25}H_{32}N_{10}O_8 \cdot 2.5H_2O$: C, 46.51; H, 5.78; N, 21.70. Found: C, 46.31; H, 5.37; N, 21.46. After drying at 100 °C immediately before the analysis the compound contained 1.5 mol of H_2O. Anal. Calcd: C, 47.84; H, 5.62; N, 22.32. Found: C, 47.58; H, 5.22; N, 22.31.

1,4-Di(adenosin-N^6-yl)butane (IIIc)′ and N^6-(4-Aminobutyl)adenosine (IVb). A. From I and IIb. A solution of chloro nucleoside I (0.99 g, 3.45 mmol), 1,4-diaminobutane (IIb, 0.88 g, 10 mmol), and triethylamine (2.08 ml, 15 mmol) in DMF (5 ml) was kept at room temperature for 20 h. The mixture containing precipitated triethylamine hydrochloride was evaporated to dryness at room temperature and 0.1 mm. The solid residue was dissolved in 50% ethanol and the solution was applied to a Dowex 1X 2, 200–400 mesh column (HCO_3^- form, 25 ml) which was eluted with 50% ethanol (500 ml).[28] This eluate was evaporated to a white solid which was dissolved in 50% ethanol (100 ml) and the solution was applied to a Dowex 50 column (H^+ form, 40 ml). The column was eluted with 50% ethanol (1 l.) and then with 5% pyridine (1 l.). The latter eluate afforded, after evaporation in vacuo, a solid which was filtered off after addition of acetone (10 ml), 0.21 g (21%) of IIIb, mp 202–204 °C dec, ninhydrin negative; TLC (S_3) showed the presence of a trace of faster moving impurity. This material (0.1 g) was crystallized from 50% ethanol to give 50 mg of IIIc: mp 228–232 °C dec; on TLC (S_3) homogeneous; $[\alpha]^{20}D$ −19.2° (*c* 0.5, CH_3SOCH_3); UV max (H_2O) 264 nm (ε 30 800); (0.01 N HCl) 264 nm (ε 38 100); NMR (CD_3SOCD_3) δ 8.27 (s, 2, H_8), 8.15 (s, 2, H_2), 5.87 (d, 2, $H_{1'}$, $J_{1',2'}$ = 6 Hz), 5.33 and 5.07 (poorly resolved m, disappeared on addition of D_2O, 6, OH), the rest of the ribose bands (including *N*-methylene groups) are not well resolved between 3.6–4.7, 1.67 (poorly resolved m, 4, CCH_2). Anal. Calcd for $C_{24}H_{32}N_{10}O_8 \cdot 2.5H_2O$: C, 45.49; H, 5.89; N, 22.11. Found: C, 45.55; H, 5.63; N, 22.27. After drying at 100 °C immediately before the analysis, the compound contained 1.25 mol of H_2O. Anal. Calcd: C, 47.17; H, 5.69; N, 22.92; H_2O, 3.55. Found: C, 47.13; H, 5.43; N, 23.01; H_2O, 3.82.

The Dowex 50 column was eluted further with 0.5 and 3% aqueous ammonia (1 l. each). Evaporation of the effluents gave only a very little of the UV-absorbing material; therefore the elution continued with 3% NH_3 in 50% ethanol (1 l.). After evaporation of the eluate, product IVb was obtained (0.91 g, 78%), mp 186 °C, ninhydrin positive and TLC (S_3) homogeneous. An analytical sample was crystallized from ethanol: mp 191–192 °C; $[\alpha]^{25}D$ −52.8° (*c* 0.5, CH_3SOCH_3); UV max (95% ethanol) 269 nm (ε 16 800); (0.01 N HCl) 264 nm (ε 19 300); NMR (CD_3SOCD_3) δ 8.23 (s, H_8, 1), 8.10 (s, 1, H_2) 5.84 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6 Hz), 3.5–4.5, the rest of the ribose protons, OH's and NCH_2– are not well resolved, ca. 1.5 (poorly resolved m, 8, 2 protons disappeared on addition of D_2O, CCH_2 and NH_2). Anal. Calcd for $C_{14}H_{22}N_6O_4 \cdot H_2O$: C, 47.18; H, 6.79; N, 23.58. Found: C, 47.23; H, 6.62; N, 23.47. After drying at 100 °C immediately before the analysis the compound lost 1 mol of H_2O. Anal. Calcd: C, 49.69; H, 6.55; N, 24.84; H_2O, 5.06. Found: C, 49.47; H, 6.52; N, 24.62; H_2O, 4.53.

B. From I and IVb. A solution of chloro nucleoside I (1 g, 3.5 mmol), compound IVb (0.585 g, 1.64 mmol), and triethylamine (0.61 ml, 4.4 mmol) in DMF (20 ml) was stirred at room temperature for 4 days. After cooling (0 °C) the triethylamine hydrochloride was filtered off and the filtrate evaporated in vacuo. The residue was suspended in 50% ethanol, absorbed on Dowex 50 (H^+), and added to the column of the same resin (final column volume was 70 ml). The column was washed at 0 °C (cold room) with 50% ethanol (1 l.). Evaporation of the eluate recovered crude I (0.5 g, 50%). Elution with 5% pyridine in 50% ethanol (1 l.) gave after evaporation of the eluate bridged nucleoside IIIc (0.37 g, 36% yield). Finally, the column was washed with 3% NH_3 in 50% ethanol (1 l.) to recover IVb (0.12 g, 21%).

N^6-Ethyladenosine (VIIa). A solution of chloro nucleoside I (0.2 g, 0.70 mmol), ethylamine hydrochloride (0.114 g, 1.4 mmol), and triethylamine (0.49 ml, 3.5 mmol) in DMF (1.5 ml) was kept at room temperature for 2 days. The reaction mixture containing precipitated triethylamine hydrochloride was evaporated to dryness at room temperature and 0.1 mm. The residue was crystallized from methanol to give 150 mg (73%) of VIIa: mp 191–193 °C (lit.[21] 191–192 °C); $[\alpha]^{25}D$ −29.8° (*c* 0.5, CH_3SOCH_3); UV max (H_2O) 267 nm (ε 17 800); (0.01 N HCl) 263 nm (ε 18 600); NMR (CD_3SOCD_3) δ 8.23 (s, 1, H_8), 8.12 (s, 1, H_2), 7.65 (poorly resolved t, disappeared on addition of D_2O, 1, NH), 5.85 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6 Hz), ca. 5.2 (m, 3, disappeared on addition of D_2O, OH), the rest of the ribose proton signals and NCH_2 at δ 3.4–4.6 are not well resolved, 1.18 (t, 3, CH_3). Anal. Calcd for $C_{12}H_{17}N_5O_4 \cdot H_2O$: C, 46.00; H, 6.11; N, 22.35. Found: C, 45.93; H, 6.07; N, 22.29. After drying at 150 °C in vacuo immediately before the analysis an anhydrous compound was obtained. Anal. Calcd: C, 48.80; H, 5.81; N, 23.72; H_2O, 5.75. Found: C, 48.66; H, 5.77; N, 23.57; H_2O, 5.91.

N^6-Butyladenosine (VIIb) was prepared in analogy to VIIa from chloro nucleoside I (0.2 g, 0.70 mmol), 1-aminobutane (0.14 ml, 1.4 mmol), and triethylamine (0.29 ml, 2.1 mmol) in DMF (1.5 ml). After evaporation, the residue was crystallized from methanol to give 0.16 g (71%) of VIIb: mp 171–173 °C (lit.[21] 176 °C); $[\alpha]^{25}D$ −36.4° (*c* 0.5, CH_3SOCH_3); UV max (H_2O) 268 nm (ε 17 600); (0.01 N HCl) 264 nm (ε 17 900); NMR (CD_3SOCD_3) δ 8.22 (s, 1, H_8), 8.10 (s, 1, H_2), 5.83 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6 Hz), 7.63 (poorly resolved t, disappeared on addition of D_2O, NH), ca. 5.2 (poorly resolved m, disappeared on addition of D_2O, OH), the rest of the ribose proton signals and NCH_2 at δ 3.3–4.5 are not well resolved, ca. 1.47 and 0.88 (poorly resolved m, 7, CCH_2 and CH_3). Anal. Calcd for $C_{14}H_{21}N_5O_4$: C, 52.00; H, 6.56; N, 21.66. Found: C, 51.88; H, 6.60; N, 21.82.

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Registry No.—I, 23828-03-7; IIa, 107-15-3; IIb, 110-60-1; IIIb, 60687-64-1; IIIc, 60687-65-2; IVa, 35662-04-5; IVb, 60687-66-3; V, 39824-26-5; VI, 60687-67-4; VIIa, 14357-08-5; VIIb, 23096-10-8; ethylamine HCl, 557-66-4; butylamine, 109-73-9.

References and Notes

(1) (a) This investigation was supported in part by U.S. Public Health Service Research Grants GM-19108 and GM-21093 from the National Institute of General Medical Sciences and in part by an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit. (b) Presented in part at the 9th International Symposium on Chemistry of Natural Products, Ottawa, Canada, June 1974, Abstract, p 31B.
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Protection of Aspartic Acid, Serine, and Threonine in Solid-Phase Peptide Synthesis

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N-Boc-β-(4-bromobenzyl)aspartic acid, *N*-Boc-*O*-(4-bromobenzyl)serine, and *N*-Boc-*O*-(4-chlorobenzyl)threonine have been synthesized for use in solid-phase peptide synthesis. The side-chain protecting groups were five to seven times more stable than the parent benzyl protection in 50% trifluoroacetic acid in dichloromethane and were completely removed in liquid hydrogen fluoride at 0 °C in 10 min.

Included among recent improvements in solid-phase peptide synthesis[1] is the development of side-chain protecting groups based on quantitative measurements of their stabilities.[2,3] Since *N*-Boc protection[4,5] is commonly employed along with final deblocking in liquid hydrogen fluoride,[6] the ideal protecting group is completely stable during removal of the Boc group and completely removed in HF. We now report the synthesis and properties of three new derivatives directed toward this end.

The removal of the Boc group is effectively accomplished in 50% trifluoroacetic acid in dichloromethane.[7] Benzyl protection of the side chains of serine, threonine, and aspartic acid has been shown to be sufficiently stable in this reagent for synthesis of peptides of moderate size.[2,3] However, it would be desirable to have protecting groups for these amino acids of even greater stability. Therefore, *N*-Boc-β-(4-bromobenzyl)aspartic acid (I), *N*-Boc-*O*-(4-bromobenzyl)serine (II), and *N*-Boc-*O*-(4-chlorobenzyl)threonine (III) were synthesized with II and III isolated as dicyclohexylamine salts. All three compounds were prepared by procedures analogous to those developed for the corresponding benzyl derivatives. The steric purity of the new derivatives was assessed by two criteria.

For testing the behavior of the new protecting groups, compounds I, II, and III were converted to *N*-acetylamide derivatives (Ia, IIa, and IIIa in Table I), where any influence