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Synthesis of Actinomycin D Lactam, [1′,1′-Bis(L-*threo*-α,β-diaminobutyric acid)]actinomycin D†

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Actinomycin D lactam has been synthesized as a potential antitumor chemotherapeutic. In this peptide analog both threonine residues of actinomycin D have been replaced by L-*threo*-α,β-diaminobutyric acid. The key intermediate, N^{α}-benzyloxycarbonyl-L-*threo*-α,β-diaminobutyryl-D-valyl-L-prolylsarcosyl-L-*N*-methylvalyl(N^{β}-diaminobutyric acid) lactam (VIII), was obtained by cyclization through a *p*-nitrophenyl ester condensation between the proline and sarcosine residues. The phenoxazinone precursor, *N*-2-nitro-3-benzyloxy-4-methylbenzoyl substituent, was introduced *via* its symmetrical anhydride. [1′,1′-Bis(L-*threo*-α,β-diaminobutyric acid)]actinomycin D exhibited five times lower toxicity and antibacterial and temporary antitumor activity in mice (Ridgway Osteogenic Sarcoma) at *ca.* ten times higher dose levels than actinomycin D.

In a search for actinomycin analogs with improved chemotherapeutic properties, we are engaged in a program aimed at assessing the contribution of the peptide moiety to antitumor activity of the drug. Actinomycin D is used successfully, when combined with radiation therapy, in the treatment of Wilms' tumor.[3] However, its usefulness for other tumors is severely restricted by its toxicity.[4,5] Thus, there is strong incentive for obtaining actinomycins with broader antitumor activities and improved therapeutic indices.

All natural and biosynthetic actinomycins possess two common amino acids, the threonine residue in position 1′ and the sarcosine in position 4′. Because of this, we are interested in preparing analogs in which substitutions have been made in either of these positions and observing the biological activity of these compounds. Following a total synthesis[6] of actinomycin D, the analog [1′,1′-bis(L-α,β-diaminopropionic acid)]actinomycin D has been synthesized.[7]

In designing new peptide analogs certain empirical rules have emerged in the past; *i.e.*, functional analogs (in which chemically reactive groups have been substituted) should be isosteric and steric analogs (in which chemically nonreactive space-filling groups have been replaced) should be isofunctional.[8] Interpretations of differences in the biological properties of analogs are facilitated if minimal structural changes are made.[9]

We wish to report the synthesis of actinomycin D lactam, *i.e.*, [1′,1′-bis(L-*threo*-α,β-diaminobutyric acid)]actinomycin D (Figure 1).[1] Replacement of the L-threonine residues by L-*threo*-α,β-diaminobutyric acid affords an isosteric functional analog in which -NH- groups are substituted for the lactam oxygens of actinomycin D. A common amino acid has therefore been replaced in a natural compound by an unusual amino acid rarely found in nature. Only recently has α,β-diaminobutyric acid been identified as a constituent of amphomycin.[10] The use of unusual residues in preparing analogs of biologically active peptides has provided many interesting results, deamino-oxytocin being a classical example.[11]

The synthetic route (Figure 2) resembles that recorded for the synthesis of the diaminopropionic acid analog.[7] The pentapeptide lactam is obtained by ring closure between the proline and sarcosine residues using a *p*-nitrophenyl ester condensation. N^{α}-Benzyloxycarbonyl-L-*threo*-α,β-diaminobutyric acid methyl ester hydrochloride (I)‡,§ was condensed with *tert*-butyloxycarbonyl-L-*N*-methylvaline[1] by the mixed anhydride method.[12] The β-dipeptide methyl ester (II) was obtained in crystalline form; however, a side product was also isolated from the mother liquor after Sephadex LH-20 chromatography. This compound, characterized by nmr spectroscopy, was N^{α}-benzyloxycarbonyl-N^{β}-isobutyloxycarbonyl-L-*threo*-α,β-diaminobutyric acid methyl ester (IIa). Obviously, it resulted from nucleophilic attack by the β-amino group of the α,β-diaminobutyric acid derivative on the isobutylcarbonyl of the mixed anhydride.[13]

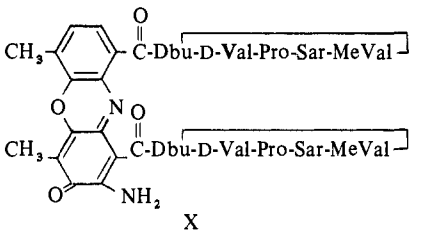
The *tert*-butyloxycarbonyl group of II was removed by the action of trifluoroacetic acid.[14] Mixed anhydride coupling of III with *tert*-butyloxycarbonylsarcosine[15] gave the crystalline tripeptide methyl ester IV. After alkaline saponification the mixed anhydride was generated and coupled to D-valyl-L-proline *p*-nitrophenyl ester[7] to afford N^{α}-benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonylsarcosyl-L-*N*-methylvalyl)-L-*threo*-α,β-diaminobutyryl-D-valyl-L-proline *p*-nitrophenyl ester (VI).

Removal of the *tert*-butyloxycarbonyl group from VI by the action of boron trifluoride in glacial acetic acid[6,16] and conversion to the hydrochloride VII was followed by cyclization at 55–60° in pyridine (c ~0.05) in the presence of triethylamine.[6,17] N^{α}-Benzyloxycarbonyl-L-*threo*-α,β-diaminobutyryl-D-valyl-L-prolylsarcosyl-L-*N*-methylvalyl-(N^{β}-diaminobutyric acid) lactam (VIII) was obtained in 38%

†Syntheses of Actinomycin and Analogs. 9. For part 8, see ref 1. Abbreviations follow the rules of the IUPAC-IUB Commission on Biochemical Nomenclature in ref 2. Dbu, L-*threo*-α,β-diaminobutyric acid.

‡E. Atherton and J. Meienhofer, unpublished results.

§Prepared from L-threonine *via* 48-hr treatment of *N,O*-ditosyl-L-threonine methyl ester with NH_3-saturated CH_3OH, followed by ester hydrolysis in 6 *N* HCl, N^{β}-acylation with *tert*-butyloxycarbonyl azide, Na in liquid NH_3 cleavage of the N^{α}-tosyl group, benzyloxycarbonylation of N^{α}, and acidolytic liberation of N^{β}.



X

Figure 1. Structure of actinomycin D lactam (X), 2-amino-4,6-dimethylphenoxazinone(3)-1,9-bis[carbonyl-L-*threo*-α,β-diaminobutyryl-D-valyl-L-prolylsarcosyl-L-*N*-methylvalyl(N^{β}-diaminobutyric acid) lactam].

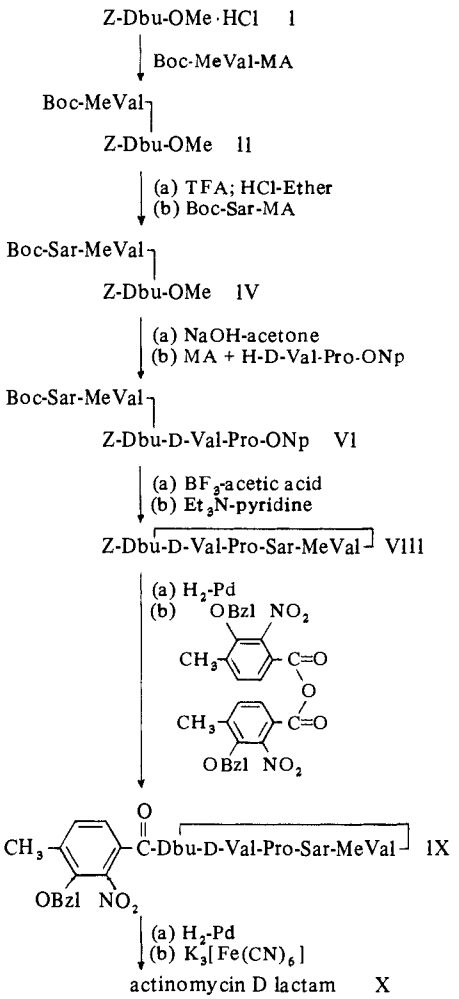


Figure 2. Schematic pathway of actinomycin D lactam synthesis: Boc, *tert*-butyloxycarbonyl; Dbu, L-*threo*-α,β-diaminobutyric acid; MA, mixed anhydride; MeVal, L-*N*-methylvaline; ONp, *p*-nitrophenyl ester; TFA, trifluoroacetic acid; Z, benzyloxycarbonyl.

yield after chromatographic purification on Sephadex LH-20. The benzyloxycarbonyl group was cleaved from VIII by palladium-catalyzed hydrogenolysis.[18,19] Introduction of the 2-nitro-3-benzyloxy-4-methylbenzoyl moiety *via* the acid chloride[20] gave a low yield of IX. The reaction was monitored by the disappearance of ninhydrin color which was very slow. When the symmetrical anhydride, generated *in situ* from 2-nitro-3-benzyloxy-4-methylbenzoic acid and dicyclohexylcarbodiimide,[21] was used, the condensation was very fast with no apparent ninhydrin reaction after 5 min at room temperature. The product was purified by Sephadex LH-20 chromatography and then crystallized to give N^{α}-(2-nitro-3-benzyloxy-4-methylbenzoyl)-L-*threo*-α,β-diaminobutyryl-D-valyl-L-prolylsarcosyl-L-*N*-methylvalyl(N^{β}-diaminobutyric acid) lactam (IX) in high yield (74%).

Hydrogenolysis of IX, to remove the benzyl ether and to reduce the nitro group to an amino group, was followed by oxidation with potassium ferricyanide.[22] Actinomycin D lactam (X) was obtained in crystalline form after Sephadex LH-20 chromatography. The yield was low (22%) in contrast to the actinomycin D preparation. This might be due to steric factors and modified reaction conditions might be required.

Microbiological assays against *Lactobacillus arabinosus* and *Lactobacillus casei*[23] indicate that ten times higher doses (ID_{50} 0.5 and 0.3-0.5 μg/ml, respectively) are required to produce the same effect as actinomycin D. The toxicity of the lactam in mice is somewhat lower than that of actinomycin D;[5] normal mice tolerate a dose of 1 mg/kg; however, the LD_{100} is reached at 2 mg/kg. Studies on the antitumor activity of the analog against AKD_2F1 male mice bearing Ridgway Osteogenic Sarcoma[24] indicate that there was little or no tumor inhibition at 300 μg/ml. At doses of 600 and 1200 μg/ml the growth rate of the tumor was temporarily suppressed during the period (4 days) of administration, but the tumors increased rapidly in size afterward. These results indicate that the analog exhibits a lower therapeutic index than actinomycin D[6] in mice bearing Ridgway Osteogenic Sarcoma. The antitumor activity is of shorter duration, which might be a consequence of weaker binding to nuclear DNA or of impaired uptake by the tumor cells. Tlc data show that the lactam is more hydrophilic than actinomycin D. Screening tests for effects against other tumors and cells in culture are in progress and will be reported elsewhere.

Experimental Section

Details on materials and methods have been described before.[6] Solvent systems for silica gel thin layer chromatography are A, *sec*-BuOH–HCOOH–H_2O (75:13.5:11.5); B, *sec*-BuOH–10% NH_4OH (85:15); and C, EtOAc–CH_3OH (85:15).

N^{α}-Benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonyl-L-*N*-methylvalyl)-L-*threo*-α,β-diaminobutyric Acid Methyl Ester (II). *tert*-Butyloxycarbonyl-L-*N*-methylvaline dicyclohexylammonium salt (4.59 g, 11.1 mmol) was suspended in EtOAc–H_2O and treated with 1 *N* H_2SO_4. The organic phase was washed twice with H_2O, dried ($MgSO_4$), and evaporated. The residual oil was dissolved in tetrahydrofuran (25 ml) and cooled to −15°. To the stirred solution *N*-methylmorpholine (1.25 ml, 11.1 mmol) and isobutyl chloroformate (1.47 ml, 11.1 mmol) were added. After stirring for 1–2 min, a precooled solution of N^{α}-benzyloxycarbonyl-L-*threo*-α,β-diaminobutyric acid methyl ester hydrochloride (I) (1.68 g, 5.56 mmol) was added in DMF (10 ml) containing Et_3N (0.78 ml, 5.56 mmol). The solution was stirred at −15° for 1.5 hr and at room temperature for 5.5 hr when it was poured into H_2O and extracted with EtOAc. The organic phase was washed with 1 *M* citric acid (three times), H_2O, 1 *N* $NaHCO_3$ (three times), and saturated NaCl (twice), dried ($MgSO_4$), and evaporated. Crystallization from CH_3OH–H_2O gave white needles: 1.7 g (64%); mp 120–122°; $[\alpha]^{20}D$ −6.3° (*c* 1, CH_3OH); tlc R_f 0.85 (A), 0.79 (B), 0.74 (C). *Anal.* ($C_{24}H_{37}N_3O_7$) C, H, N.

A side product remained in the mother liquor after crystallization. This was isolated by Sephadex LH-20 chromatography in EtOH as an oil (~20%) which gradually crystallized. The compound was identified by nmr spectroscopy in $CDCl_3$ as N^{α}-benzyloxycarbonyl-N^{β}-isobutyloxycarbonyl-L-*threo*-α,β-diaminobutyric acid methyl ester (IIa): mp 48–50°; $[\alpha]^{20}D$ +35.2° (*c* 1, CH_3OH); tlc R_f 0.45 (A), 0.53 (C); nmr ($CDCl_3$) δ 0.9 [doublet, 6 protons, $CH_2CH(CH_3)_2$], 1.2 (doublet, 3 protons, CH_3CHNH), 1.95 [multiplet, 1 proton, $CH_2CH(CH_3)_2$], 3.75 [singlet, 4 protons, $COOCH_3$ and $OCH_2CH(CH_3)_3$ with 1 proton from doublet at 3.8], 4.4 (multiplet, 2 protons, $CH_3CHCHCOOCH_3$), 4.9 (multiplet, NH), 5.13 (singlet, 2 protons, $C_6H_5CH_2O$), 5.8 (multiplet, NH), 7.35 (singlet, 5 protons, $C_6H_5CH_2O$). In the presence of CD_3–COOD the multiplets at 4.9 and 5.8 disappeared indicating exchange of nitrogen protons. *Anal.* ($C_{18}H_{26}N_2O_5$) C, H, N.

N^{α}-Benzyloxycarbonyl-N^{β}-(L-*N*-methylvalyl)-L-*threo*-α,β-diaminobutyric Acid Methyl Ester Hydrochloride (III). II (1.34 g,

2.8 mmol) was dissolved in trifluoroacetic acid (45 ml) and stirred at 0–5° for 1 hr. The trifluoroacetic acid was evaporated, the resultant oil dissolved in CH_3OH, and 4 *N* HCl in ether added (1.5 ml). After stirring for 5–10 min, the solution was evaporated and the residue dried thoroughly *in vacuo* (P_2O_5 and KOH): tlc R_f 0.60 (A), 0.42 (C).

N^{α}-Benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonylsarcosyl-L-*N*-methylvalyl)-L-*threo*-α,β-diaminobutyric Acid Methyl Ester (IV). To a stirred solution of *tert*-butyloxycarbonylsarcosine (0.79 g, 4.2 mmol) in tetrahydrofuran (20 ml), at −15°, was added *N*-methylmorpholine (0.47 ml, 4.2 mmol) followed by isobutyl chloroformate (0.56 ml, 4.2 mmol). After stirring at −15° for 1–2 min, a precooled solution was added of III (2.8 mmol) in DMF (10 ml) containing Et_3N (0.39 ml, 2.8 mmol). The solution was stirred at −15° for 1.5 hr and at room temperature for 5 hr. It was then worked up as described for II. The product crystallized partially from ether–hexane, 0.39 g. However, most of the compound remained in the mother liquor which gave a foam (0.8 g) on evaporation: total yield, 1.19 g (77%); mp 108–109°; $[\alpha]^{20}D$ −26° (*c* 1, CH_3OH); tlc R_f 0.86 (A), 0.77 (B), 0.73 (C). *Anal.* ($C_{27}H_{42}N_4O_8$) C, H, N.

N^{α}-Benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonylsarcosyl-L-*N*-methylvalyl)-L-*threo*-α,β-diaminobutyric Acid (V). IV (1.15 g, 2.09 mmol) was dissolved in acetone (10 ml). H_2O (5 ml) and 1 *N* NaOH (2.3 ml) were added. After stirring for 1 hr at room temperature, the mixture was poured into H_2O and extracted twice with EtOAc. The aqueous phase was isolated and acidified with 1 *M* citric acid, and the oil which formed was extracted into EtOAc which was washed with saturated NaCl, dried ($MgSO_4$), and evaporated yielding an oil, 0.95 g (85%), which failed to crystallize, tlc R_f 0.47 (B).

N^{α}-Benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonylsarcosyl-L-*N*-methylvalyl)-L-*threo*-α,β-diaminobutyryl-D-valyl-L-proline *p*-Nitrophenyl Ester (VI). To a stirred solution of V (0.95 g, 1.77 mmol) in tetrahydrofuran (8 ml), at −15°, was added *N*-methylmorpholine (0.20 ml, 1.77 mmol), followed by isobutyl chloroformate (0.23 ml, 1.77 mmol). After stirring for 1–2 min, a precooled (−20°) DMF solution (10 ml) of D-valyl-L-proline *p*-nitrophenyl ester hydrobromide (0.81 g, 1.95 mmol) was added, which, just prior to addition, was neutralized with Et_3N (0.27 ml, 1.95 mmol). The solution was stirred at −15° for 1.5 hr and at room temperature for 1.5 hr. Work-up as described for II gave an oil. Precipitation by adding an ether solution of the oil to stirred hexane at −60° yielded an amorphous powder: 0.9 g (60%) (further 0.2 g of oil was obtained on evaporation of the mother liquor, total yield 73%); mp 64–68°; $[\alpha]^{20}D$ −31.2° (*c* 1, CH_3OH); tlc R_f 0.66 (A), 0.66 (C). *Anal.* ($C_{42}H_{59}N_7O_{12}$) C, H, N.

N^{α}-Benzyloxycarbonyl-N^{β}-(sarcosyl-L-*N*-methylvalyl)-L-*threo*-α,β-diaminobutyryl-D-valyl-L-proline *p*-Nitrophenyl Ester Hydrochloride (VII). The pentapeptide *p*-nitrophenyl ester VI (1.05 g, 1.23 mmol) was dissolved in 0.4 *M* BF_3 in AcOH (50 ml) and stirred at room temperature for 1.5 hr. Evaporation gave an oil which was dissolved in AcOH and treated with excess 4 *N* HCl in ether. After stirring for 5–10 min, the mixture was evaporated; the residual oil was dissolved in the minimum amount of AcOH and added dropwise to vigorously stirred ether. The precipitate which formed was triturated several times with ether, filtered, and dried *in vacuo* to give an amorphous powder: 0.72 g (74%); tlc R_f 0.68 (A), 0.18 (C).

N^{α}-Benzyloxycarbonyl-L-*threo*-α,β-diaminobutyryl-D-valyl-L-prolylsarcosyl-L-*N*-methylvalyl(N^{β}-diaminobutyric acid) Lactam (VIII). VII (0.72 g, 0.91 mmol) was dissolved in DMF (10 ml) containing AcOH (0.3 ml) and added dropwise over a period of 1.5 hr to stirred pyridine (1400 ml) at 55–60° containing Et_3N (0.51 ml). Stirring was continued for an additional 3 hr. The pyridine was evaporated and the residual oil worked up as described for II using 1 *N* mineral acid in place of 1 *M* citric acid. The crude material was subjected to column chromatography on Sephadex LH-20 in EtOH. Combination and evaporation of the fractions from the fastest eluting peak gave the lactam VIII which was precipitated by adding an EtOAc solution to cold vigorously stirred hexane yielding 216 mg (38.5%): mp 145–149°; $[\alpha]^{20}D$ −25.3° (*c* 0.9, CH_3OH); tlc R_f 0.61 (A), 0.26 (C). *Anal.* ($C_{31}H_{46}N_6O_7$) C, H, N.

N^{α}-(2-Nitro-3-benzyloxy-4-methylbenzoyl)-L-*threo*-α,β-diaminobutyryl-D-valyl-L-prolylsarcosyl-L-*N*-methylvalyl(N^{β}-diaminobutyric acid) Lactam (IX). (A) Acid Chloride Method. The benzyloxycarbonyl-protected cyclic lactam VIII (87.4 mg, 0.14 mmol) was dissolved in CH_3OH (15 ml) containing 0.4 ml of 4 *N* HCl in ether and hydrogenolyzed in the presence of freshly prepared Pd black for 1 hr. Tlc in system C indicated complete removal of the benzyloxycarbonyl group. The catalyst was filtered off through prewashed Celite and the solution evaporated. Ether was removed several times under vacuum and the residual foam dried thoroughly. After drying, the cyclic lactam was dissolved in a minimum amount of DMF and cooled to 0°. 2-Nitro-3-benzyloxy-4-methylbenzoyl chloride (175 mg, 0.57 mmol) was added with stirring followed by triethylamine (1 mmol). The mixture was allowed to warm to room temperature. After stirring for 1 hr the mixture was poured into H_2O, extracted with EtOAc, and worked up and purified as described for VIII. Combination of the chromatographic fractions comprising the fastest eluting peaks gave the required product in 56% yield.

(B) Symmetrical Anhydride Method. The cyclic lactam derivative VIII (120 mg, 0.19 mmol) was hydrogenated and treated as described in A. 2-Nitro-3-benzyloxy-4-methylbenzoic acid (230 mg, 0.8 mmol) was dissolved in EtOAc (6 ml) and a solution of dicyclohexylcarbodiimide (78.5 mg, 0.38 mmol) added in EtOAc (5 ml). The solution was warmed slightly and allowed to stir for 15 min at room temperature. Then a solution of hydrogenolized cyclic lactam in DMF (5 ml) containing *N*-methylmorpholine (0.18 ml, 1.6 mmol) was added dropwise from a pipet. The mixture was stirred for 30 min and *N*-methylmorpholine (0.05 ml) added. After working up as described above, the compound was isolated and crystallized from EtOAc by the addition of hexane to give 110 mg (77%): mp 164–168°; $[\alpha]^{20}D$ −35.3° (*c* 1, CH_3OH); tlc R_f 0.64 (A), 0.63 (B). *Anal.* ($C_{38}H_{51}N_7O_9$) C, H, N.

Actinomycin D Lactam, 2-Amino-4,6-dimethylphenoxazinone-(3)-1,9-bis[carbonyl-α,β-L-*threo*-diaminobutyryl-D-valyl-L-prolylsarcosyl-L-*N*-methylvalyl(N^{β}-diaminobutyric acid) Lactam (X). Cyclic IX (165 mg, 0.22 mmol) was dissolved in CH_3OH (20 ml) and hydrogenated in the presence of Pd black for 1.5 hr. The catalyst was removed by filtration through prewashed Celite and the CH_3OH solution added to an equal volume of 0.067 *M* phosphate buffer at pH 7.1 containing K_3FeCN_6 (195 mg, 0.59 mmol). The pH was maintained at 7.1 by the addition of 0.067 *M* KH_2PO_4 and the solution stirred for 20 min at room temperature. It was then partitioned between EtOAc and H_2O and the aqueous phase extracted with EtOAc. The EtOAc solutions were combined and washed with 1 *M* $NaHCO_3$ (twice), 1 *M* H_2SO_4 (twice), and saturated NaCl (twice), dried (Na_2SO_4), and evaporated. The crude product was chromatographed on Sephadex LH-20 using EtOH as eluent. Fractions comprising the first large peak were combined and evaporated. After drying the lactam analog was obtained in crystalline form from EtOAc–CH_3OH–hexane: 31 mg (22%); mp 260–267°; $[\alpha]^{20}D$ −206.5 ± 3° (*c* 0.19, CH_3OH); tlc R_f 0.27 (A), 0 (C); uv max (CH_3OH) 238 nm (ε 34,556), 443 (23,309). *Anal.* ($C_{62}H_{88}N_{14}O_{14}\cdot H_2O$) C, H, N.

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Platelet Aggregation Inhibitors. 4.[1] N^6-Substituted Adenosines

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Various N^6-substituted adenosines **1-21** were synthesized in 20-100% yields by reaction of 6-chloropurine ribonucleoside or 2-amino-6-chloropurine ribonucleoside with the requisite substituent amine in EtOH. N^6-Benzyl- (or allyl-) adenosine 5′-monophosphate **27** and **28** was prepared by treatment of adenosine 5′-monophosphate with benzyl (or allyl) bromide and alkali. Among the N^6-substituted adenosines **1-26** and N^6-substituted adenosine 5′-monophosphate **27** and **28**, N^6-phenyl- (**1**), N^6-*o*-chlorophenyl- (**2**), N^6-*p*-chlorophenyl- (**4**), N^6-*p*-methoxyphenyl- (**7**), N^6-cyclopentyl- (**10**), N^6-cyclohexyl- (**11**), N^6-(*p*-hydroxyphenylethyl)- (**23**), N^6-(indole-3-ethyl)- (**24**), and N^6-allyl- (**26**) adenosines showed strong inhibitory activity at 10^{-4} *M* against adenosine 5′-diphosphate and collagen induced rabbit platelet aggregation. Incubation of these active compounds with rabbit platelet-rich plasma for a period exceeding 2 hr did not lead to loss of activity. Rabbit platelet adhesiveness to glass beads was inhibited by compounds **1** and **11**. These also inhibited adenosine 5′-diphosphate induced platelet aggregation in plasmas obtained from rabbits given single intravenous doses of 8 mg/kg. Compounds **1** and **11** were also active as inhibitors of human platelet aggregation.

Platelet aggregation or platelet thrombus formation is of primary importance in arterial thrombogenesis.[2] Agents that inhibit platelet aggregation are of interest as potential drugs, and a number of compounds have been tested as inhibitors of adenosine 5′-diphosphate (ADP) and/or collagen induced platelet aggregation.[3-14] Among them, prostaglandin E_1,[4] certain adenosine derivatives,[5-7] pyrimidopyrimidines,[8,9] thieno compounds,[10] thiazolo compounds,[11] [(dialkylamino)-alkyl]thio heterocyclic compounds,[12,13] and a fluorene derivative[14] are typical strong inhibitors of platelet aggregation, but few have been evaluated as antithrombotic agents owing to their undesirable side effects or toxicity.

Among the derivatives of adenosine, 2-chloroadenosine[5] has been found to be a powerful inhibitor of platelet aggregation, but it is very toxic.[15] Recently, 2-methylthioadenosine 5′-monophosphate which is less toxic has been evaluated to be an antithrombotic agent, although it showed rather weak inhibitory activity against platelet aggregation.[16] In the course of our studies on platelet aggregation inhibitors, we have found 6-hydroxyaminopurine ribonucleosides to be ten times as potent as adenosine in the inhibition of rabbit plasma platelet aggregation.[7] However, they showed little activity against human platelet aggregation.[7,17]

In order to secure further information on the structure–activity relationships in this series of adenosine derivatives, some additional N^6-substituted adenosines and N^6-substituted adenosine 5′-monophosphates were prepared and examined for the inhibitory activity of platelet aggregation induced by ADP and collagen.

Chemistry. 6-Substituted purine ribonucleosides have been synthesized by treatment of 6-halogenopurine ribonucleosides,[7,18-22] 6-methyl- (or benzyl-) thiopurine ribonucleosides,[23] or 6-trimethylsilyloxypurine ribonucleosides[24] with appropriate amines or *via* N^1-substituted adenosines obtained by reaction of adenosine with appropriate halides.[21,25] Fleysher, *et al.*,[20-22] have synthesized several N^6-substituted adenosines by treatment of 6-chloropurine ribonucleoside with amines in the presence of $CaCO_3$ or Et_3N and also by the N^1 quaternization of adenosine with the appropriate halides. In the present study, 6-chloropurine ribonucleoside[18] or 2-amino-6-chloropurine ribonucleoside[26] was allowed to react with large excesses of various amines and the corresponding N^6-substituted adenosines **1-21** were obtained. The reaction was performed in EtOH in the absence of an auxiliary acid acceptor which facilitated the isolation of the products. The structures and the physical data of **1-21** are given in Table I. Compounds **8-10** and **12-21** have not been reported thus far, while compounds **1**,[21] **11**,[22] **22**,[18,27] **25**,[21] and **26**[20] have been reported in the literature and compounds **2-7** have been described in the patent field.[28] N^6-Substituted adenosine 5′-monophosphates **27** and **28** were prepared by reaction of adenosine 5′-monophosphate (AMP) with benzyl (or allyl) bromide and subsequent treatment of the intermediate N^1-benzyl- (or allyl-) AMP with alkali (Scheme I).

Pharmacology. The N^6-substituted adenosines **1-26** and N^6-substituted AMP **27** and **28** were tested *in vitro* as inhibitors of ADP- and collagen-induced rabbit platelet aggregation according to the method of Born and Cross.[29] The