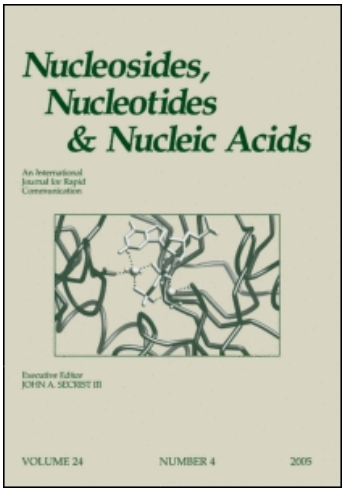

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Synthesis of Potential Purinoceptor Antagonists: Application of P1-tBu Phosphazene Base for Alkylation of Adenine in Solution and on Solid Phase

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SYNTHESIS OF POTENTIAL PURINOCEPTOR ANTAGONISTS: APPLICATION OF P1-*t*BU PHOSPHAZENE BASE FOR ALKYLATION OF ADENINE IN SOLUTION AND ON SOLID PHASE

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□ *Alkylation of adenine in solution and on solid phase was accelerated by phosphazene base P1-tBu compared to mineral bases. The reactions in solution afforded regioselectively the appropriate N9-alkylated adenines with high preparative yields while the reaction with polystyrene resin-bound N-bromoacetylated peptides gave three regioisomers (alkylated at the N9, N7, and N3 position of adenine) in a 4:2:1 molar ratio. Ten novel nonphosphate nucleotide analogues were tested in an ADP-induced platelet aggregation assay.*

Keywords Adenine alkylation; Phosphazene base; Nucleotide analog; Solid phase synthesis

INTRODUCTION

Adenine nucleosides and nucleotides bind to intracellular proteins, which participate in important functions such as signalling, metabolism, and cell proliferation. These adenine derivatives also play important roles in the extracellular milieu where they interact with almost 20 different membrane receptor proteins, purinoceptors, and an unknown number of ectoenzymes including kinases and phosphatases.[1] Structure-activity studies have shown that nucleotide-selective P2X and P2Y receptors require that the ligands contain a negatively charged region (the polyphosphate chain) concentrated in close proximity to the base moiety. Small differences in the number of

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negative charges or the spatial arrangement of these pharmacophore elements may cause complete loss of affinity or dramatic changes in pharmacological activity of the ligand from an agonist to an antagonist. Sequence conservation of positively charged amino acids in P2 nucleotide receptors supports the idea of important electrostatic interactions between the receptor and the negative charges of the polyphosphate moiety of nucleotides.[2] Recently, acyclic nucleotide analogues containing no sugar moiety have been synthesized and tested as ligands of nucleotide receptors.[2,3] Remarkable antagonistic activity has been demonstrated for several of these compounds. We and others have shown that phosphate groups of nucleotides can be replaced with nonhydrolyzable carboxylate groups in antagonists of P2 nucleotide receptors.[4–6] To combine two latter approaches we report here the development of synthetic methods for preparation of acyclic nonphosphate nucleotide analogues.

Derivatization of adenine has been an important strategy for the development of selective ligands for the regulation of the activity of pharmacologically important proteins. The alkylation in neutral medium gives mostly N3-substituted products while the reaction of adenine anion leads to N9 and N7 derivatives.[7] Mineral hydrides and carbonates are the most frequently used bases for the activation of adenine in alkylation reactions, giving more or less regioselectively and with moderate yields the N9 derivatives, the most common in biological systems. Due to low solubility of adenine and its metal salts in organic solvents these reactions require long times or high temperatures to proceed. Thus, these reagents are not well applicable for solid phase synthesis where good swelling of the polymer resin and sufficient solubility of the reagent in the same solvent are prerequisites for the exploitation of an organic reaction.

The recent use of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for the alkylation of adenine in dimethylformamide (DMF) led to improvements in yield and regioselectivity of the reactions.[8] Inasmuch as the basicity of DBU is insufficient for full deprotonation of adenine in non-hydroxylic solvents dimethyl sulfoxide (DMSO), DMF, and acetonitrile ($pK_a = 14.2$ for adenine[9] and $pK_{BH+} = 13.9$ for DBU[10] in DMSO), excess of the base, higher temperatures, and longer reaction times are required for quantitative alkylation of adenine. The propensity of DBU to alkylation under these conditions limits its use as an ionizing base for the synthesis on solid phase.

Schwesinger phosphazene bases[11] have been successfully used in different applications both in solution (including alkylation of derivatives of 2-aminopurine[12]) and on a solid phase.[13] As a special class of strong organic nonionic bases, these compounds possess some useful characteristics for practical applications: their basicity can be varied over a wide pK_a range, they are not prone to alkylation, and their cationic forms are well soluble in nonhydroxylic solvents. Commercial availability of phosphazenes with different basicity (well established in physicochemical studies[14]) makes it possible

to deprotonate the reagent selectively and quantitatively at the most acidic site in the presence of the equimolar amount of the base.

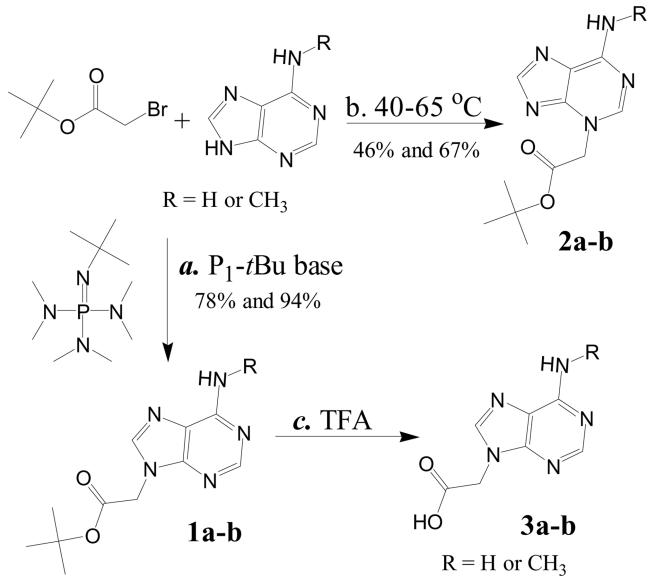
Adenine-9-acetic acid is a building block of several biologically active structures like peptide nucleic acid (PNA) monomers[15] and P2Y receptor antagonists.[16] Derivatives of adenine-9-acetic acid are usually prepared by alkylation of adenine with an ester or an amide of halogenoacetic acid in a basic medium and further transformation of the intermediates to the desired products. The present study describes the application of a phosphazene base for the solubilization and deprotonation of adenine, which leads to substantial acceleration of the reaction of adenine with bromoacetylated substrates in solution and to the first successful application of adenine alkylation reaction on solid phase. P1-*t*Bu phosphazene [*tert*-butylimino-*tris*(dimethylamino)phosphorane] was chosen as the ionizing base proceeding from its suitable basicity characteristics ($pK_{BH+} = 15.7$ in DMSO[10]): only small excess of the base is sufficient for complete deprotonation of adenine. The method was used for the synthesis of nucleotide analogues, derivatives of adenine with different positioning of negatively charged carboxylate groups. The newly synthesized compounds were tested as potential inhibitors of platelet aggregation promoted by the activation of adenine nucleotide receptors.

RESULTS AND DISCUSSION

Addition of phosphazene P1-*t*Bu to the suspension of purines in DMF resulted in complete solubilization of N6-methyladenine and partial solubilization of adenine. The following reaction with *t*-butyl bromoacetate (Scheme 1) was completed within seconds. Esters of adenine-9-acetic acids **1a** and **1b** were isolated as single N9-regioisomers with high yields (78% and 94%). The conversion of the starting adenines in the presence of the same molar quantity of DBU was less effective. Alkylations with NaH were slower and resulted in **1a** and **1b** with preparative yields 55% and 81%, respectively.

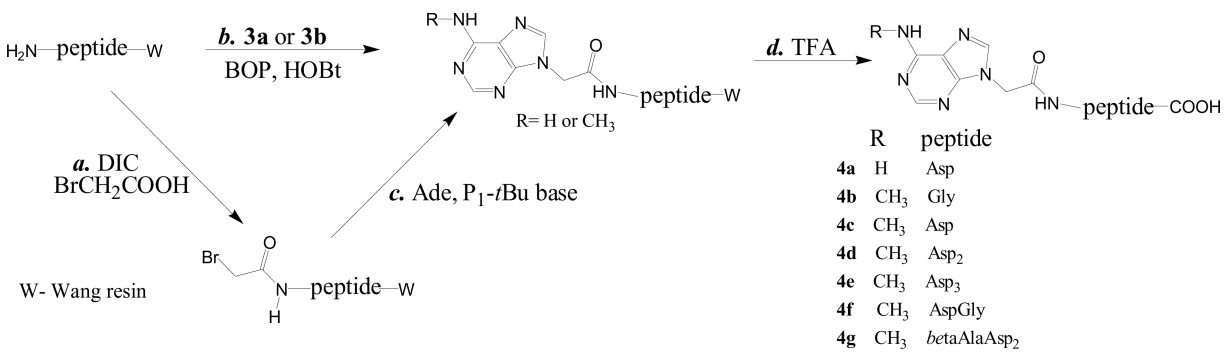
Reactions under the conditions where no base was added required higher temperatures (40–65°C, 2–6 h) to proceed and gave expectedly[7] N3-substituted derivatives **2a–b** (**2a** 46%, **2b** 67%) as the main products. Only traces of the N9-isomer could be detected. The reaction in the presence of diisopropylethylamine (trialkylamines are approximately 4–5 pK_a units weaker bases than DBU and adenine anion in DMSO[9]) yielded a complicated mixture of products at 70°C in 2 h. **1a–b** were treated with trifluoroacetic acid (TFA) to remove *t*-butyl groups and the obtained adenine-9-acetic acids (**3a–b**) were used in further reactions.

Adenine-9-acetic acid was attached to peptides *via* two different approaches (Scheme 2): either **3a** and **3b** were reacted with polymer-bound peptides or the peptide was first bromoacetylated by the previously described procedure,[17] which was followed by the reaction with adenine anion in



SCHEME 1 Reagents and conditions: (a) P1-*t*Bu phosphazene base, DMF, RT. (b) DMF, 40–65°C, 2–6 h. (c) TFA.

DMF in the presence of P1-*t*Bu phosphazene base. Finally, the products were cleaved from the Wang resin and the protection groups were removed with 95% TFA. The alkylation of adenine on solid support was quick and gave high yields, but the reactions produced mixtures of regioisomers (N9-, N7-, and N3-alkylated products in a 4:2:1 molar ratio, Figure 1). The application of DBU afforded worse solubilization of adenines and gave lower yields of alkylation of adenine due to the reaction of DBU with bromoacetylated peptide. The molar ratio of the obtained adenine regioisomers was similar in the case of both applied bases. The separation of the target 9-regioisomer by C18 reverse-phase HPLC (elution with water-acetonitrile gradient in the presence of 0.1% TFA) was successful for only some of the peptide conjugates (e.g., **4c**). Better separation of the isomers was achieved in the presence of acetate buffer (AcOH/AcONa, pH = 5.0–5.5) where three peaks could be distinguished for acetyl-glycine derivative of N6-methyl-adenine (**4b**). The adenine moiety of the N3-alkylated derivatives is protonated at this pH while those of N7 and N9 derivatives are predominately neutral.[7] The position of adenine alkylation was established on the basis of analysis of NMR and UV spectra of the compounds.[18] Absorption maximums in the UV spectra of the chromatographically separated isomers at pH 5.5 were the following: 266 nm (N6-MeAde-9-AcGly) **4b**, 278.5 nm (N6-MeAde-7-AcGly), and 283 nm (N6-MeAde-3-AcGly). Two rotamers related to C6-N6 bonds could be detected by the NMR spectra of N3-, N6-disubstituted adenines (**2b** and N3-isomers of **4b–d**).[19]



SCHEME 2 Reagents and conditions: (a) Bromoacetic acid, DIC, DMF. (b) Adenine-9-acetic acid, BOP, HOBt, DMF. (c) Adenine, P1-*t*Bu phosphazene base, DMF. (d) TFA.

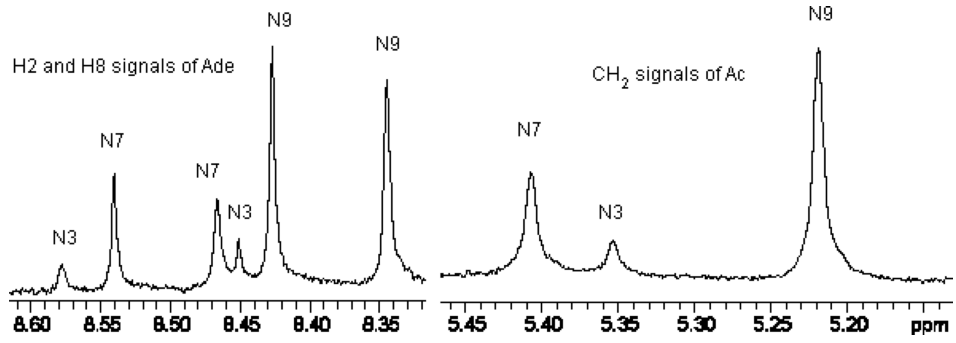


FIGURE 1 Fragments from ^{1}H NMR (D_2O) spectrum of the product of alkylation of adenine on solid phase (aspartic acid derivative of adenine acetic acid **4a**). The signals of three regioisomers (N9-alkyl, N7-alkyl, and N3-alkyl) are visible.

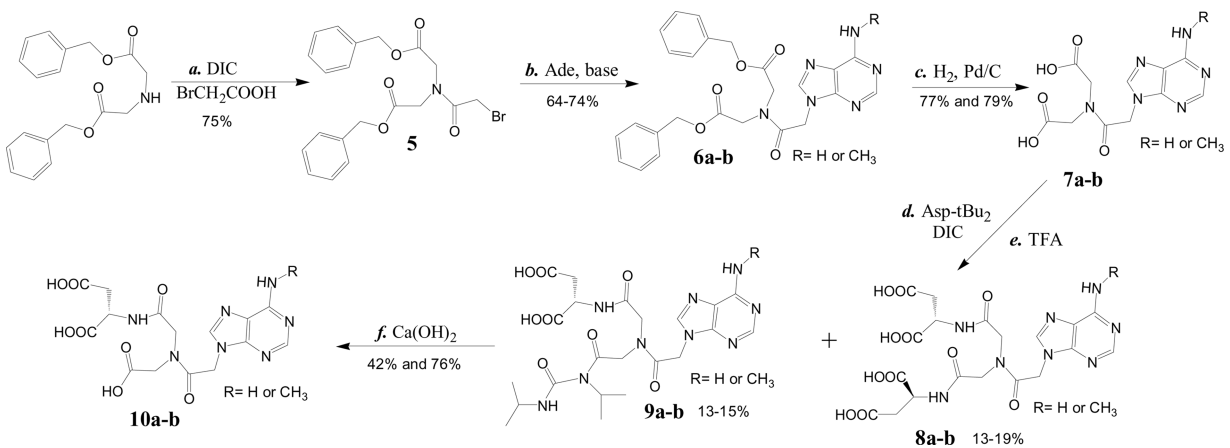
Derivatives of adenine-9-acetic acid containing two or more carboxylate moieties were synthesized according to Scheme 3. Bromoacetylation of dibenzyl iminodiacetate[20] in the presence of diisopropylcarbodiimide (DIC) led to the amide **5**. Reactions of **5** with adenine or N6-methyladenine in the presence of bases (NaH, K_2CO_3, or phosphazene P1-*t*Bu base) resulted in adenine derivatives **6a–b**. The application of the organic base gave a higher yield and the reaction was completed in a shorter time. The following hydrogenation of dibenzyl esters **6a–b** on Pd/C led to diacids **7a–b** that were coupled with di-*t*-butyl ester of l-aspartic acid using DIC activation. The products were treated with TFA and, after removal of the solvent, were purified by HPLC. Two pairs of individual compounds could be separated (**8a–b** and **9a–b**). DIC activation without auxiliary reagents like 1-hydroxybenzotriazole was obviously not an appropriate method for derivatives iminodiacetic acid as it gave low yields. Compounds **9a–b** were N-acylurea derivatives of **7a–b** that had formed as side products during the activation of carboxylate groups with the carbodiimide. The removal of the N-acylurea moiety from **9a–b** by hydrolysis in $Ca(OH)_2$ solution[21] yielded **10a–b**, derivatives of adenine with three carboxylate groups.

The new acyclic nonphosphate nucleotide analogues were evaluated for activity in human platelet aggregation assay. Compounds **4e**, **7a**, **8a**, and **10b** produced a small inhibition of ADP-induced platelet aggregation (Table 1) mediated via $P2Y_1$ and $P2Y_{12}$ receptors. The mechanism of the inhibitory effect of these compounds has not yet been determined.

EXPERIMENTAL

Materials and Methods

^{1}H and ^{13}C NMR spectra were taken on Bruker AC 200P spectrometer. Masses of all synthesized compounds were measured with MALDI-TOF



SCHEME 3 Reagents and conditions: (a) bromoacetic acid, DIC, dichloroethane (DCE). (b) Adenine or N6-methyladenine, NaH or phosphazene base, DMF. (c) H_2 Pd/C in EtOH or BuOH/AcOH. (d) L-Aspartic acid di-*t*-butyl ester, DMF, DIC, 0–20°C. (e) TFA. (f) $Ca(OH)_2$, 6 h.

TABLE 1 Inhibition of Platelet Aggregation (Conditions in Experimental)

Compound	Inhibition (%) of platelet aggregation at 100 μM ($N = 3$)
4g, **7b**	NE
4c, **4d**, **8b**, **10a**	NR
4e	28.7 ± 8.1
7a	11.7 ± 6.0
8a	9.7 ± 4.8
10b	53.3 ± 3.3

NE = No effect, NR = small and not reproducible effect, N = number of measurements.

mass spectrometer (built at the National Institute of Chemical Physics and Biophysics, Tallinn, Estonia). UV spectra were taken on Specord M40 (Carl Zeiss, Jena) or Unicam UV 300 (ThermoSpectronic) spectrometers. Reactions were monitored by thin-layer chromatography (TLC) on POLYGRAM® SIL G/UV_{254} plates. UV light was used for the visualization of the products. Langford Sonomatic® 375H ultrasonic bath was used for sonication. Column chromatography was performed on silica gel 60 (0.04–0.063 mm) from Fluka. The final products were purified with Gilson HPLC system using C18 reverse-phase column (GL Sciences) Inertsil ODS-3 (5 μm, 25 $\times$ 0.46 cm) with monitoring at 260 nm. Elution was performed with water-acetonitrile gradient (0.1% TFA), flow rate 1 mL/min. The separated products were freeze-dried. All compounds used in biological tests were >95% pure by HPLC (detected at 260 nm, Table 2). Adenine, diisopropylethylamine (DIEA), and *t*-butyl bromoacetate were purchased from Sigma; celite from Aldrich; derivatives of amino acids and other chemicals used in peptide synthesis were from Advanced ChemTech and Nova Biochem; acetone from Merck; DBU, DMF, bromoacetic acid, sodium hydride, N6-methyladenine, P1-*t*Bu phosphazene, and DIC from Fluka. All other HPLC grade solvents were from Rathburn Ltd. Derivatives of L-aspartic acid were used for the preparation of adenine-peptide conjugates.

Biological Testing of Compounds

Blood was collected from healthy volunteers into syringes containing 1/6 final blood volume of anti-coagulant ACD (65 mM citric acid, 85 mM sodium citrate, 110 mM dextrose). Washed platelets were prepared as previously described.[22] Briefly, ACD-anticoagulated blood was centrifuged at 275 g for 16 min, the platelet-rich plasma was collected and centrifuged at 2200 g for 13 min. The supernatant was removed and the platelet pellet resuspended in Tyrode's buffer containing 0.35% bovine serum albumin and 0.05 U/mL potato apyrase. Platelet aggregation was measured using the optical mode of a ChronoLog aggregometer (Havertown, PA). Five hundred microliters of

TABLE 2 MS and HPLC Data

Compound	Nr	Formula	MW_{calc} (g/mol)	MS M + H	RT min	HPLC purity^{260nm} (%)
AdeAcAsp	**4a**	$C_{11}H_{12}N_6O_5$	308.25	309	10.4	98
NMeAdeAcGly	**4b**	$C_{10}H_{12}N_6O_3$	264.24	265	13.2	98
NMeAdeAcAsp	**4c**	$C_{12}H_{14}N_6O_5$	322.28	323	13.3	96
NMeAdeAcAsp2	**4d**	$C_{16}H_{19}N_7O_8$	437.36	438	13.1	98
NMeAdeAcAsp3	**4e**	$C_{20}H_{24}N_8O_{11}$	552.45	553	13.6	97
NMeAdeAcAspGly	**4f**	$C_{14}H_{17}N_7O_6$	379.33	380	15.4	99
NMeAdeAcbeetaAlaAsp2	**4g**	$C_{19}H_{24}N_8O_9$	508.44	509	16.5	97
$BrAcIdaBn_2$	**5**	$C_{20}H_{20}BrNO_5$	434.28	434	—	>92[&]
$AdeAcIdaBn_2$	**6a**	$C_{25}H_{24}N_6O_5$	488.5	489	—	>92[&]
$NMeAdeAcIdaBn_2$	**6b**	$C_{26}H_{26}N_6O_5$	502.52	503	—	>92[&]
AdeAcIda	**7a**	$C_{11}H_{12}N_6O_5$	308.25	309	9.3	96
NMeAdeAcIda	**7b**	$C_{12}H_{14}N_6O_5$	322.28	323	11.5	96
AdeAcIda(Asp)Asp	**8a**	$C_{19}H_{22}N_8O_{11}$	538.43	539	12.7	98
NMeAdeAcIda(Asp)Asp	**8b**	$C_{20}H_{24}N_8O_{11}$	552.45	553	15.5	99
N-acylurea derivative	**9a**	$C_{22}H_{31}N_9O_8$	549.54	550	29.7	95
N-acylurea derivative	**9b**	$C_{23}H_{33}N_9O_8$	563.56	564	32.8	96
AdeAcIdaAsp	**10a**	$C_{15}H_{17}N_7O_8$	423.34	424	11.3	99
NMeAdeAcIdaAsp	**10b**	$C_{16}H_{19}N_7O_8$	437.36	438	14.6	95

MW_{calc} = calculated molecular weight.

MS M + H = protonated monoisotopic peak in MALDI-TOF mass spectrum.

RT min = HPLC retention time in minutes using C18 reversed phase column Inertsil ODS-3, 5 μm, 25 × 0.46 cm (GL Sciences) with UV detection at 260 nm. Elution was performed with water-acetonitrile linear gradient (flow rate 1ml/min) from 0% to 30% acetonitrile over 40 min.

[&] = purity estimated by ^{1}H NMR.

platelet suspension at a density of 5×10^8 platelets/mL containing 1 mg/mL fibrinogen were warmed to 37°C and stirred at 1000 rpm. The effect of inhibitors on platelet aggregation was studied by preincubating the platelet suspension with the indicated concentration of the inhibitor for 2 min prior to the addition of a maximally effective concentration of ADP (usually 1–5 μM, determined for each blood donor from concentration-effect curve).

Synthesis

***t*-Butyl Adenine-9-acetate (1a).** Adenine (27.2 mg, 0.20 mmol) and phosphazene base P1-*t*-Bu (65 μL, 0.24 mmol; Fluka) were suspended in DMF (2.5 mL). The suspension was stirred while bromoacetic acid *t*-butyl ester (30 μL, 20.3 mmol) was added. A clear solution formed within seconds and the solution was evaporated to dryness. The crude mixture was dissolved in EtOAc and washed with a solution of KH_2PO_4/K_2HPO_4. Column chromatography ($CHCl_3$/MeOH10 : 2, $R_f = 0.6$) yielded **1a** (39.3 mg, 77.7%). Adenine (50 mg, 0.37 mmol) and 60% NaH (19.5 mg, 0.49 mmol) were suspended in DMF (1.5 mL). The mixture was stirred for 30 min and then bromoacetic acid *t*-butyl ester (66 μL, 0.45 mmol) was added. The stirring was continued overnight and the solvent was removed in *vacuo*. The product

was purified by column chromatography ($CHCl_3$/MeOH 10:2) to give **1a** (50.6 mg, 55%). ^{1}H NMR ($CDCl_3$)δ1.49(9H, *s*, *t*Bu), 4.87(2H, *s*, CH_2), 5.80 (2H, br, NH_2), 7.87 and 8.36 (2H, s, A2 and A8). ^{13} C NMR ($CDCl_3$) δ28.2 (3C, *t*Bu), 45.1 (CH_2), 83.7 (*t*Bu), 119.4 (A5), 141.1 (A8), 150.5 (A4), 153.4 (A2), 155.7 (A6), 166.3 (COO).

***t*-Butyl N6-Methyladenine-9-acetate (1b).** N6-methyladenine (37.5 mg, 0.25 mmol) and phosphazene base P1-*t*-Bu (81 μL, 0.32 mmol) were dissolved in DMF (1 mL). Bromoacetic acid *t*-butyl ester (45 μL, 0.30 mmol) was added and the solution was stirred for 1 h. The solvent was removed under reduced pressure. The crude mixture was dissolved in EtOAc and washed with solution of KH_2PO_4/K_2HPO_4. The residue was purified by column chromatography ($CHCl_3$/MeOH 10:1) to give **1b** (62 mg, 94%). N6-Methyladenine (19.1 mg, 0.128 mmol) and 60% NaH (7.0 mg, 0.175 mmol) were suspended in DMF (0.75 mL). The mixture was stirred for 30 min and then bromoacetic acid *t*-butyl ester (23 μL, 0.156 mmol) was added. The stirring was continued overnight and the solvent was removed in *vacuo*. The product was purified by column chromatography ($CHCl_3$/MeOH 10:2) to give **1b** (27.2 mg, 81%). ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s, *t*Bu), 3.20 (3H, br, NCH_3), 4.85 (2H, s, CH_2), 6.02 (1H, br, NH), 7.80 and 8.40 (2H, s, A2 and A8). ^{13}C NMR ($CDCl_3$)δ27.9(NCH_3), 28.2 (3C, *t*Bu), 45.0 (CH_2), 83.6 (*t*Bu), 119.6 (A5), 140.3 (A8), 149.5 (A4), 153.6 (A2), 155.8 (A6), 166.4 (COO).

***t*-Butyl Adenine-3-acetate (2a).** Suspension of adenine (40.5 mg, 0.30 mmol) and bromoacetic acid *t*-butyl ester (55 μL, 0.37 mmol) in DMF (2.1 mL) was stirred for 2 h at 65°C. The solvent was removed under reduced pressure and the residue was partitioned between EtOAc and aqueous K_2CO_3. The organic solution was collected and dried. The residue was purified by column chromatography ($CHCl_3$/MeOH 10:2, $R_f = 0.6$) to give **2a** (34.2 mg, 46%). ^{1}H NMR (CD_3SOCD_3)δ1.41 (9H, s, *t*Bu), 5.09 (2H, s, CH_2), 8.03 (2H, br, NH_2), 7.75 and 8.32 (2H, s, A2 and A8). ^{13}C NMR (CD_3SOCD_3)δ27.5 (3C, *t*Bu), 49.9 (CH_2), 82.2 (*t*Bu), 119.9 (A5), 144.0 (A2), 149.9 (A4), 152.3 (A8), 154.9 (A6), 166.3 (COO).

***t*-Butyl N6-Methyladenine-3-acetate (2b).** Suspension of N6-methyladenine (65 mg, 0.44 mmol) and bromoacetic acid *t*-butyl ester (98 μL, 0.66 mmol) in DMF (3.5 mL) was stirred for 7 h at 40°C. The solvent was removed under reduced pressure. The residue was purified by column chromatography ($CHCl_3$/MeOH 10:2, $R_f = 0.7$) to give **2b** (100.6 mg HBr salt, 67%). ^{1}H NMR ($CDCl_3$)δ1.44 (9H, s, *t*Bu), 3.22 (3H, br, NCH_3), 5.01 (2H, s, CH_2), 7.96 and 8.00 (2H, s, A2 and A8), 9.42 (1H, br, NH). ^{13}C NMR ($CDCl_3$)δ27.8 (NCH_3), 28.1 (3C, *t*Bu), 50.3 (CH_2), 84.0 (*t*Bu), 121.2 (A5), 143.3 (A2), 149.2 (A4), 153.0 (A8), 154.6 (A6), 165.7 (COO).

Synthesis of AdeAcAsp (4a), NMeAdeAcGly (4b), NMeAdeAcAsp (4c), and NMeAdeAcAsp$_2$ (4d). Wang-type peptide synthesis resin, Fmoc-Asp(*t*-Bu)-resin (0.7 mmol/g; Advanced ChemTech) or Fmoc-Gly-resin (0.87 mmol/g; Advanced ChemTech), was swollen in DMF. Fmoc protection was removed by double treatment of the resin with 20% piperidine in DMF (5 + 15 min). The following L-aspartic acids (in the form of Fmoc-Asp[*t*-Bu]) were attached to the resin with BOP/HOBt activation (0.95 eq.) in the presence of N-methylmorpholine (5%, v/v) in DMF. The Fmoc protection of the N-terminal amino group was removed by the double treatment with 20% piperidine in DMF (5 + 15 min). The resin-bound peptides or amino acids were bromoacetylated by adding the solution of 1.2 M bromoacetic acid (3 eq.) and DIC (3 eq.) in DMF to the resin. The mixture was agitated for 1 h at RT and the resin was washed with DMF. N6-methyladenine (1.5 eq) and phosphazene base P1-*t*-Bu (1.5–1.7 eq) were dissolved in DMF (final concentration 0.2–0.4 M) and added to the resin. The mixture was shaken for 1 h and washed with DMF and DCE. Adenine-peptide conjugates were cleaved from the resin and the protection groups were removed by 2 h treatment with 95% TFA. Mixtures of three regioisomers of adenine (N9-alkyl, N7-alkyl, and N3-alkyl with ratios of about 4:2:1) were obtained. The products were purified on a C18 reversed phase column with water-ACN gradient (0.1% TFA). Freeze-drying gave the adenine-peptide conjugates in the form of white solids with 50–60% yields (mixtures of regioisomers alkylated at the N9, N7, and N3 positions of adenine in 4:2:1 molar ratio by NMR).

Synthesis of NMeAde-9-AcAsp$_2$ (4d), NMeAde-9-AcAsp$_3$ (4e), NMeAde-9-AcAspGly (4f), and NMeAde-9-AcβAlaAsp$_2$ (4g). Wang-type peptide synthesis resin, Fmoc-Asp(*t*-Bu)-resin (100 mg, 0.7 mmol/g; Advanced ChemTech) or Fmoc-Gly-resin (100 mg, 0.87 mmol/g; Advanced ChemTech), was swollen in DMF. Fmoc protection was removed by treatment of the resin with 20% piperidine in DMF (5 + 15 min). The following L-aspartic acids (in the form of Fmoc-Asp[*t*-Bu]) or β-alanine (Fmoc-β-Ala) were attached to the resin with BOP/HOBt activation (0.95 eq.) in the presence of N-methylmorpholine (5%, v/v) in DMF. The Fmoc protection of the N-terminal amino group was removed by the double treatment with 20% piperidine in DMF (5 + 15 min). N-Me-Ade-9-Ac (**3b**; 3 eq., compared to N-terminal amino groups) was suspended in DMF in the presence of DIEA (5%, v/v). BOP and HOBt (both 0.95 eq.) were added to the suspension. After 3 min, preactivation the solution was poured on the resin. The reaction vessel was shaken at room temperature for 1 h. Adenine-peptide conjugates were cleaved from the resin and the protection groups were removed by 2 h treatment with 95% TFA. The products were purified on a C18 reverse-phase column with water-ACN gradient (0.1% TFA). Freeze-drying gave the adenine-peptide conjugates in the form of white solids.

Ade-9-AcAsp (4a). ^{1}H NMR (D_2O) δ 2.99 (2H, d, $J = 5.9$ Hz, β Asp), 5.22 (2H, s, CH_2 Ac), 8.34 and 8.43 (2H, s, A2 and A8), signals of α Asp were hidden by HDO.

NMeAde-9-AcGly (4b). ^{1}H NMR (CD_3SOCD_3)δ3.03 (3H, s, NCH_3), 4.55 (2H, d, $J = 5.6$ Hz, α Gly), 5.01 (2H, s, CH_2 Ac), 8.29 and 8.38 (2H, s, A2 and A8), 8.75 (1H, t, $J = 5.6$ Hz, C(O)NH), 9.0 (1H, br, NH A).

NMeAde-9-AcAsp (4c). ^{1}H NMR (CD_3SOCD_3)δ2.67 (1H, dd, $J = 16.9$ and 6.4 Hz, β Asp), 2.69 (1H, dd, $J = 16.9$ and 5.8 Hz, β Asp), 3.03 (3H, s, NCH_3), 4.55 (1H, ddd, $J = 8.0$, 6.4 and 5.8 Hz, α Asp), 4.99 (2H, s, CH_2 Ac), 8.27 and 8.37 (2H, s, A2 and A8), 8.83 (1H, d, $J = 8.0$ Hz, C(O)NH), 8.95 (1H, br, NH A). ^{13}C NMR (CD_3SOCD_3)δ27.9 (NCH_3), 36.0 (β Asp), 45.0 (CH_2 Ac), 48.8 (α Asp), 118.1 (A5), 142.8 (A8), 148.7 (2C, A2 and A4), 152.1 (A6), 165.8 (C(O)NH), 171.3 and 171.7 (2C, COOH).

NMeAde-9-AcAsp$_2$ (4d). ^{1}H NMR (CD_3SOCD_3) δ 2.4–2.8 (4H, m, β Asp), 3.00 (3H, s, NCH_3), 4.45–4.7 (2H, m, α Asp), 4.93 (2H, s, CH_2 Ac), 8.19 and 8.32 (2H, s, A2 and A8), 8.28 (1H, d, $J = 8.2$ Hz, NH Asp), 8.26 (2H, br, NH_2), 8.76 (1H, d, $J = 8.0$ Hz, NH Asp). ^{13}C NMR (CD_3SOCD_3) δ28.3 (NCH_3), 35.8 and 36.3 (2C, β Asp), 45.0 (CH_2 Ac), 48.6 and 49.4 (2C, α Asp), 118.3 (A5), 142.2 (A8), 149.9 (2C, A4 and A2), 153.1 (A6), 166.0 and 170.0 (2C, C(O)NH), 171.3, 171.4 and 171.9 (3C, COOH).

NMeAde-9-AcAsp$_3$(4e). ^{1}H NMR of Na salt (D_2O) δ 2.42–2.85 (6H, m, β Asp), 3.08 (3H, NCH_3), 4.35 (1H, dd, $J = 8.5$ and 4.9 Hz, α Asp), 4.63–4.75 (2H, m, α Asp), 5.94 (2H, s, CH_2 Ac), 8.08 and 8.20 (2H, s, A2 and A8).

NMeAde-9-AcAspGly (4f). ^{1}H NMR of Na salt (D_2O) δ2.64 (1H, dd, $J = 16.2$ and 8.7 Hz, β Asp), 2.76 (1H, dd, $J = 16.2$ and 4.9 Hz, β Asp), 3.05 (3H, s, NCH_3), 3.75 and 3.77 (2H, d, $J^2 = 17.3$ Hz, α Gly), 4.72 (1H, dd, $J = 8.7$ and 4.9 Hz, α Asp), 4.91 (2H, s, CH_2 Ac), 8.03 and 8.14 (2H, s, A2 and A8). ^{13}C NMR of Na salt (D_2O) δ28.1 (NCH_3), 39.5 (β Asp), 44.1 (α Gly) 45.9 (CH_2 Ac), 52.6 (α Asp), 119.1 (A5), 142.8 (A8), 148.8 (A4), 153.5 (A2), 155.9 (A6), 169.3 and 173.4 (2C, C(O)NH), 176.8 and 178.3 (2C, COO-).

NMeAde-9-Ac βAlaAsp$_2$ (4g). ^{1}H NMR (CD_3SOCD_3) δ2.30 (2H, t, $J = 7.1$ Hz, CH2 β Ala), 2.37–2.76 (4H, m, β Asp), 3.01 (3H, s, NCH_3), 3.28 (2H, m, $J = 2 \times 7.1$ and 5.5 Hz, βAla), 4.45–4.68 (2H, m, α Asp), 4.89 (2H, s, CH_2 Ac), 8.13 (1H, d, $J = 8.1$ Hz, C(O)NH), 8.23 and 8.34 (2H, s, A2 and A8), 8.27 (1H, d, $J = 8.3$ Hz, C(O)NH), 8.36 (1H, t, $J = 5.5$ Hz), 8.80 (1H, br, NH A). ^{13}C NMR (CD_3SOCD_3) δ28.0 (CH_3, 34.9, 35.4, 35.8, and 36.0 (4C, βAla and β Asp), 45.1 (CH_2 Ac), 48.5, and 49.1 (2C, α Asp), 118.3

(A5), 142.6 (A8), 149.2 (2C, A2 and A4), 152.6 (A6), 165.8, 170.2, and 170.4 (3C, C(O)NH), 171.5 (2C, COOH), 172.0 (COOH).

N-(Bromoacetyl) iminodiacetic Acid Dibenzylester (5). BrAcIdaBn$_2$. Iminodiacetic acid dibenzyl ester (161 mg, 0.514 mmol) was dissolved in DCE (0.5 mL) and solution of bromoacetic anhydride (synthesized separately *in situ* by coupling with DIC) (146 mg, 0.56 mmol) in DCE (1.8 mL) was added. The solution was stirred for 1 h and then the solvent was removed. The product **5** (168.5 mg, 75% yield) was separated by column chromatography ($CHCl_3$/EtOAc, 4:1, $R_f = 0.75$). ^{1}H NMR ($CDCl_3$) δ3.86 (2H, s, CH_2Br), 4.25 and 4.27 (4H, s, CH_2N), 5.15 and 5.19 (4H, s, CH_2Ph), 7.3 - 7.4 (10H, m, Bn). ^{13}C NMR ($CDCl_3$) δ25.4 (CH_2Br), 48.8 and 51.2 (2C, CH_2N), 67.3 and 67.9 (2C, CH_2Ph), 128.4–129.1 (10C, Bn), 135.0 and 135.4 (2C, Bn1), 167.4 (C(O)N), 168.3 and 168.6 (2C, COO).

N-(Adenine-9-yl-acetyl)iminodiacetic Acid Dibenzylester (6a). AdeAcIdaBn$_2$. Adenine (10.5 mg, 0.078 mmol) and 60% NaH (2.3 mg, 0.058 mmol) were suspended in DMF (0.5 mL). The mixture was stirred for 30 min and then BrAcIdaBn$_2$ (**5**) (28 mg, 0.064 mmol) in DMF (0.9 mL) was added. The stirring was continued overnight and the solvent was removed in *vacuo*. The mixture was acidified with acetic acid and purified by column chromatography ($CHCl_3$ and $CHCl_3$/MeOH, 11:2, $R_f = 0.6$) to give **6a** (23.4 mg, 74% yield). ^{1}H NMR ($CDCl_3$) δ4.26 and 4.36 (4H, s, CH_2N), 5.06 (2H, s, CH_2 Ac), 5.13 and 5.19 (4H, s, CH_2Ph), 5.63 (2H, br, NH_2), 7.25–7.5 (10H, m, Bn), 7.86 and 8.32 (2H, s, A2 and A8). ^{13}C NMR ($CDCl_3$) δ43.8 (CH_2 Ac), 49.2 and 50.5 (2C, CH_2N), 67.5 and 68.1 (2C, CH_2Ph), 118.6 (A5), 128.2–129.4 (10C, Bn), 134.9 and 135.2 (2C, Bn1), 141.5 (A8), 150.1 (A4), 152.8 (A2), 155.8 (A6), 167.1 (C(O)N), 168.2 and 168.4 (COO).

N-(N6-Methyladenine-9-yl-Acetyl)iminodiacetic Acid Dibenzylester (6b). NMeAdeAcIdaBn$_2$. N6-methyladenine (23.3 mg, 0.156 mmol) and 60% NaH (7.2 mg, 0.18 mmol) were suspended in DMF (0.95 mL). The mixture was stirred for 30 min and then BrAcIdaBn$_2$ (56.5 mg, 0.130 mmol) (**5**) in DMF (0.3 mL) was added. The stirring was continued overnight. Acetic acid (60 μL) was added and the solvents were removed in vacuo. The product was purified by column chromatography ($CHCl_3$ and $CHCl_3$:MeOH, 10:2. $R_f = 0.68$) to give **6b** (41.8 mg, 64% yield). N6-methyladenine (22.2 mg, 0.149 mmol) and phosphazene base P1-*t*-Bu (42 μL, 0.165 mmol) were dissolved in DMF (0.4 mL). The solution was added to BrAcIdaBn$_2$ (53.9 mg, 0.124 mmol) and stirred for 1 h. The solvent was evaporated to dryness. The crude mixture was partitioned between EtOAc and an aqueous solution of KH_2PO_4/K_2HPO_4. The product **6b** (43.5 mg, 70% yield) was purified by

column chromatography ($CHCl_3$ and $CHCl_3$/MeOH, 10:1. $R_f = 0.60$). ^1H NMR ($CDCl_3$)δ3.20 (3H, br, NCH_3) 4.25 and 4.36 (4H, s, CH_2N), 5.04 (2H, s, CH_2 Ac), 5.12 and 5.17 (4H, s, CH_2Ph), 5.95 (1H, br, NH), 7.25–7.5 (10H, m, Bn), 7.80 and 8.37 (2H, s, A2 and A8). ^{13}C NMR δ27.9 (NCH_3), 43.7 (CH_2 Ac), 49.1 and 50.5 (2C, CH_2N), 67.4 and 68.0 (2C, CH_2Ph), 119.4 (A5), 128.3–129.2 (10C, Bn), 134.9 and 135.3 (2C, Bn1), 140.6 (A8), 149.3 (A4), 153.4 (A2), 155.8 (A6), 167.4 (C(O)N), 168.3 and 168.5 (COO).

N-(Adenine-9-yl-acetyl)iminodiacetic Acid (7a). AdeAcIda. AdeAcIdaBn$_2$ (23 mg, 0.053 mmol) was dissolved in EtOH (3 mL). Pd/C as a catalyst was added and hydrogen was passed through the solution until the starting material had completely disappeared. The mixture was filtered through celite and the solvent was removed (12.6 mg, 77% yield). ^{1} H NMR (CD_3SOCD_3) δ 4.03 and 4.39 (4H, s, NCH_2), 5.26 (2H, CH_2 Ac), 8.29 and 8.38 (2H, s, A2 and A8), 8.72 (2H, br, NH_2). ^{13}C NMR (CD_3SOCD_3) δ44.4(CH_2 Ac), 48.4 and 49.2 (2C, NCH_2), 117.4 (A5), 144.6, 145.2, 148.9, and 150.5 (4C, A2, A4, A6 and A8), 166.6 (C(O)N), 169.7 and 170.0 (2C, COOH).

N-(N6-Methyladenine-9-yl-acetyl)iminodiacetic Acid (7b). NMeAdeAcIda. NMeAdeAcIdaBn$_2$ (41 mg, 0.081 mmol) was dissolved in n-butanol (3.6 mL) and acetic acid (2.1 ml). Pd/C (33 mg) was added and hydrogen was bubbled through the solution for 3.5 h. The mixture was filtered through celite and the solvents were removed (20.7 mg, 78.7% yield). ^1H NMR (CD_3SOCD_3) δ3.01 (3H, s, CH_3), 4.03 and 4.39 (4H, s, NCH_2), 5.23 (2H, CH_2 Ac), 8.20 and 8.34 (2H, s, A2 and A8), 8.80 (1H, br, NH).

Coupling of N-(Adenine-9-yl-acetyl)iminodiacetic Acid (7a) and Aspartic Acid Di-tert-butyl Ester with N,N′-Diisopropylcarbodiimide (DIC). AdeAcIda (**7a**) (17.9 mg, 58 μmol) and aspartic acid di-*t*-butyl ester (35 mg, 142.7 μmol) were dissolved in DMF (0.6 mL). The solution was cooled to 0°C and DIC (20 μL, 129 μmol) was added. The solution was stirred overnight at 0–4°C and then for 10 h at room temperature. The solvents were removed and the mixture was purified by column chromatography ($CHCl_3$/MeOH, 10:2). The fraction with $R_f = 0.55$–0.60 was collected and the solvents were removed. The residue was treated with 95% TFA for 1–3 h and then the solvents were removed. The separation of the mixture by HPLC gave AdeAcIda(Asp)Asp **8a** (4.0 mg, 12.8% yield) and AdeIdaAcIda(Asp)N-acyl urea of DIC **9a** (4.6 mg, 14.4% yield). (**8a**) ^1H NMR (CD_3SOCD_3) δ 2.5–2.87 (4H, m, β Asp), 3.99 and 4.28 (4H, s, NCH_2), 4.48–4.71 (2H, m, α Asp), 5.20 (2H, s, CH_2 Ac), 8.16 and 8.29 (2H, s, A2 and A8), 8.51 (1H, d, $J = 8.0$ Hz, C(O)NH), 8.99 (1H, d, $J = 7.9$ Hz, C(O)NH).

Coupling of N-(N6-Methyladenine-9-yl-acetyl)iminodiacetic Acid (7b) and Aspartic Acid Di-tert-butyl Ester with N,N′-Diisopropylcarbodiimide (DIC). NMeAdeAcIda (**7b**) (20.7 mg, 64.2 μmol) and aspartic acid di-*t*-butyl ester (58.8 mg, 240 μmol) were dissolved in DMF (0.5 mL). The solution was cooled to 0°C and DIC (25 μL, 161 μmol) was added. The mixture was stirred for 20 h at 0°C and then for 12 h at 10–12°C. The solvent was removed and the mixture was purified by column chromatography ($CHCl_3$, $CHCl_3$/MeOH, 15:2), the fraction with $R_f = 0.5$–0.6 was collected and the solvents were removed. The residue was treated with 95% TFA for 3h. The solvents were removed and the separation of the mixture by HPLC gave NMeAdeAcIda(Asp)Asp **8b** (8.7 mg, 19.3% yield) and NMeIdaAcIda(Asp)N-acyl urea of DIC **9b** (4.9 mg, 13.5% yield). (**8b**) ^{1}H NMR (CD_3SOCD_3) δ 2.5–2.86 (4H, m, β Asp), 3.00 (3H, s, NCH_3), 3.99 and 4.28 (4H, s, NCH_2), 4.49–4.70 (2H, m, α Asp), 5.19 (2H, s, CH_2 Ac), 8.10 and 8.31 (2H, s, A2 and A8), 8.50 (1H, d, $J = 8.0$ Hz, C(O)NH), 8.62 (1H, br, NHMe), 8.98 (1H, d, $J = 8.1$ Hz, C(O)NH). ^{13}C NMR (CD_3SOCD_3) δ27.7 (NCH_3), 35.8 and 36.0 (2C, β Asp), 43.8 (CH2 Ac), 48.4 and 48.7 (2C, α Asp), 50.1 and 50.8 (2C, CH_2 Ac), 118.2 (A5), 142.2 (A8), 148.5 (A4), 149.7 (A2), 153.1 (A6), 167.3, 167.9 and 167.9 (3C, amides), 171.2, 171.4, 171.76 and 171.82 (4C, COOH).

Hydrolysis of N-Acylureas. A derivative of N-acylurea (**9a** and **9b**) was dissolved in the suspension of $Ca(OH)_2$ (5 mg of the compound in 1 mL of a suspension). The suspension was initially mixed by sonication and then stirred at room temperature for 7–9 h. The mixture was acidified with 1 M HCl and evaporated to dryness. The products were purified by HPLC. Yields: **10a** 42% and **10b** 76%. Slow rotations of unsymmetrical secondary amide bonds of the compounds give unequal signals from two rotamers into the NMR spectra. The resonances of the major rotamers are labeled with asterisks.

AdeAcIdaAsp (**10a**) ^{1}H NMR (CD_3SOCD_3) δ 2.5–2.85 (2H, m, β Asp), 4.00* and 4.02 (2H, s, NCH_2), 4.30* and 4.33 (2H, s, NCH_2), 4.47–4.70 (1H, m, α Asp), 5.16 and 5.21* (2H, s, CH_2 Ac), 7.80 (2H, br, NH_2), 8.09* and 8.11 (1H, s, A), 8.21 (1H, s, A), 8.40 and 8.76* (1H, d, $J = 8.0$ Hz, NH). NMeAdeAcIdaAsp (**10b**) ^{1}H NMR (CD_3SOCD_3) δ 2.5–2.85 (2H, m, β Asp), 3.02 (3H, br, NCH_3), 3.99* and 4.02 (2H, s, NCH_2), 4.30* and 4.34 (2H, s, NCH_2), 4.47–4.71 (1H, m, α Asp), 5.20 and 5.26* (2H, s, CH_2 Ac), 8.17 (1H, br s, A), 8.34 (1H, s, A), 8.40 and 8.77* (1H, d, $J = 8.3$ Hz and 8.0 Hz*, NH). ^{13}C NMR (CD_3SOCD_3) δ27.9 (1C, br, NCH_3), 35.8* and 36.0 (β Asp), 43.98* and 44.04 (1C, CH_2 Ac), 48.36–49.27 (2C, NCH_2), 50.5 (α Asp), 118.2 (A5), 142.5 (A8), 149.5 (2C, A2, and A4), 152.8 (A6), 167.1 and 167.3*, 167.5 and 167.7* (2C, amides), 170.1* and 170.3, 171.3 and 171.4* (2C, COOH), 171.9 (1C, COOH).

CONCLUSIONS

We have demonstrated the effectiveness of P1-*t*Bu phosphazene base for the deprotonation and alkylation of adenine. The reactions of adenine anion with bromoacetic acid derivatives were fast in solution and on solid phase and adenine-peptide conjugates could be prepared in good yields. Unexpectedly, the alkylation reactions on polystyrene Wang resin led to a mixture of regioisomers that were difficult to separate with reverse-phase HPLC. Some of the prepared adenine derivatives containing negatively charged carboxylate groups inhibited platelet aggregation caused by the activation of adenine nucleotide receptors with ADP.

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