Synthesis of 3-(2-Hydroxy-1-phenylethyl)- and 3-(2-Hydroxy-2-phenylethyl)adenine, DNA Adducts Derived from Styrene

Jan Krouželka, Igor Linhart* and Tomáš Tobrman

Department of Organic Chemistry, Institute of Chemical Technology, Prague, Technicka 1905, CZ-166 28 Prague, Czech Republic; E-mail: linharti@vscht.cz

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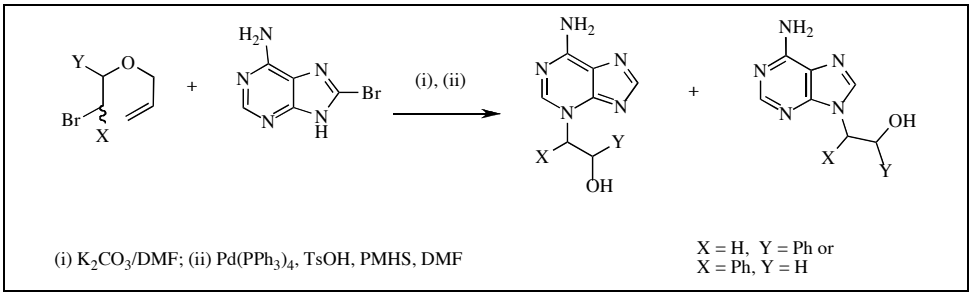

3-(2-Hydroxy-2-phenylethyl)- and 3-(2-hydroxy-1-phenylethyl)adenine, DNA adducts derived from styrene, along with their 9-substituted analogues were prepared by alkylation of 8-bromoadenine with corresponding allyl-protected bromohydrins followed by a new deallylation procedure using tetrakis(triphenylphosphine)palladium catalyzed reductive cleavage by poly(methylhydrosiloxane) in the presence of *p*-toluenesulphonic acid. This novel procedure proved to be useful for purine derivatives, which were resistant to other deallylation protocols. Structure of positional isomers was assigned using 2D NMR experiments HMBC and HMQC.

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INTRODUCTION

Alkyl-, aryl- and aralkyladenines play an important role as bioactive compounds [1] and tools for the detection and evaluation of DNA lesions [2,3]. Among them, N3 substituted adenines are nucleobase DNA adducts formed by covalent binding of electrophiles to adenine in the DNA which are cleaved off spontaneously from the DNA strand and finally excreted in urine. Therefore, they can serve as urinary biological markers of exposure to industrial and environmental mutagens and genotoxic carcinogens for early diagnosis of damage to DNA [2]. For styrene 7,8-oxide (**1**), a reactive electrophilic metabolite of styrene, N3 adenine adducts comprised 4 % of the total alkylation of DNA when double stranded DNA was alkylated at physiological pH, *i.e.*, N3 adenine adducts are the second most abundant DNA adducts derived from styrene [4]. However, no N3 adenine adducts were formed by the reaction of **1** with 2'-deoxy-adenosine-3'-phosphate [4], adenosine [5] or 2'-deoxy-adenosine [6]. Therefore, unlike other styrene derived DNA adducts, N3 adenine adducts are not accessible by direct reactions of styrene 7,8-oxide with nucleosides or nucleotides.

Adenine itself reacts with alkylating agents in the presence of a base rather non-selectively to yield a mixture of products alkylated at various nitrogens, N9 being usually the most prominent site of attack [7-9].

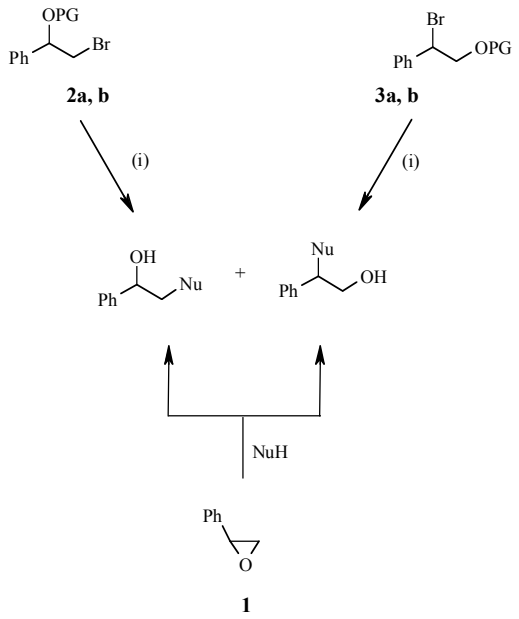
In this study we describe a synthesis of both N3 and N9 substituted adenines derived from **1**, the former ones as biomarkers of DNA damage caused by styrene exposure, the latter as suitable internal standards for the development of analytical methods to determine adenine adducts in urine. Because the primary aim was to obtain pure samples of products to be used as analytical standards we focused on the product purity rather than to strive for maximum yields.

RESULTS AND DISCUSSION

Styrene 7,8-oxide is a weak electrophile reacting with nucleophiles non-selectively at both α- and β-position giving rise to two isomeric products. In our previous work we found that allyl and benzyl protected bromohydrins **2a,b** and **3a,b** can be used as suitable synthetic equivalents of **1** to achieve selective alkylation (Scheme 1) [10,11]. In addition, benzyl-protected triflate 2-benzyloxy-2-phenylethyl triflate (**4**) [12] was tested as a more reactive alkylating reagent.

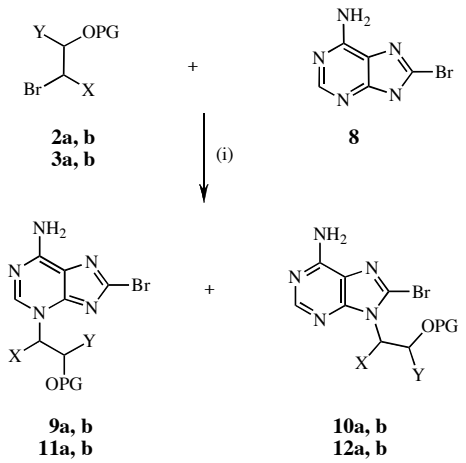
Although in the presence of a base, the alkylation of adenine is directed mainly to the N9 position [7] a selective alkylation at N3 was observed when the alkylation of adenine by reactive allylic or benzylic halides was performed in dimethylacetamide and no base except adenine itself was employed [13]. However, our attempts to alkylate adenine with allyl protected bromohydrins **2a** and **3a** at the same reaction conditions led to a mixture of at least four products as detected by

Scheme 1



PG = allyl or benzyl; (i) NuH followed by deprotection

Scheme 2



2, 9, 10 X=H, Y=Ph **a**, PG = allyl
3, 11, 12 X=Ph, Y=H **b,** PG = benzyl

(i) K_2CO_3/DMF, 50 - 100°C

TLC and ^{1}H-NMR. Adenine itself could be alkylated in DMF at 125°C with non-protected 2-bromo-1-phenylethanol (**2**) in the presence of 18-crown-6 and potassium carbonate as a base yielding 29 % of 9-(2-hydroxy-2-phenylethyl)adenine (**5**) as the main and only isolated product. At least two by-products were detected by TLC.

Isomeric bromohydrin, 2-bromo-2-phenylethanol (**3**) did not react with adenine under the same reaction conditions but its allyl- and benzyl-protected derivatives **3a** and **3b** each gave a mixture of alkylated adenines of which corresponding protected 9-(2-hydroxy-1-phenyl-ethyl)- and 7-(2-hydroxy-1-phenylethyl)adenines (**6a,b** and **7a,b**, respectively) were isolated.

To prepare both N3 and N9 substituted adenines we decided to use 8-bromoadenine (**8**) as a precursor of adenine, which after being alkylated can be converted easily to the adenine moiety by catalytic reduction of bromine. In a recent study, alkylation of **8** by 4-fluorobenzyl bromide gave N3- and N9-substituted products in nearly 1:1 ratio [14]. The alkylations of **8** with **2a**,**b** and **3a**,**b** (Scheme 2) were carried out in DMF using potassium carbonate as a base at temperatures between 50 and 100°C.

The results are summarized in the Table 1. The alkylation proceeded at N3 and N9 position of the purine moiety giving two products, the N3 substituted derivatives being the major ones. Alkylation with **2a** and **2b** gave a mixture of two products, *i.e.*, allyl- or benzyl-protected 8-bromo-3-(2-hydroxy-2-phenylethyl)adenine (**9**) and 8-bromo-9-(2-hydroxy-2-phenylethyl)adenine (**10**).

Table 1

Alkylation of 8-Bromoadenine (**8**)

Reagent	t [°C]	Reaction time [h]	Product ratio 3N:9N[a]	Yield [%] 3N + 9N
2a	70	4[b]	85 : 15	45
2a	70	20	71 : 30	39
2a	100	20	67 : 33	55
2b	70	16	80 : 20	25
2b	70	72	65 : 35	57
2b	100	20	60 : 40	69
3a	50	48	67 : 33	80
3a	70	24	62 : 38	90
3a	90	5	65 : 35	62
3b	50	18	55 : 45	62
3b	70	7	60 : 40	63
3b	90	5	60 : 40	65
4	r.t.	4	40 : 60	84

[a] Product ratios were determined by integration of the C(2)*H* signals in the NMR spectra. [b] The ratio of the reactants **2a** : **8** was 6 : 1 in this experiment.

The product ratio was temperature dependent, lower temperatures and shorter reaction times gave a better selectivity for the N3 alkylated products (Table 1). 2-Benzyloxy-2-phenylethyl triflate (**4**) [12] showed a higher reactivity than bromides but it gave predominantly the N9 substituted product **10b** the ratio of **9b**:**10b** being 2:3.

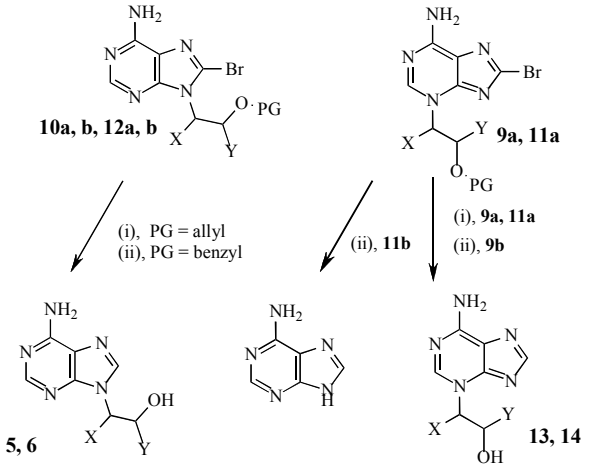
Protected bromohydrins **3a** and **3b** gave also corresponding N3 and N9 substituted products, *i.e.*, allyl- or benzyl-protected 8-bromo-3-(2-hydroxy-1-phenyl-ethyl)adenine (**11a,b**) and 8-bromo-9-(2-hydroxy-2-phenyl-ethyl)adenine (**12a,b**). Unlike for the phenethyl

bromides **2a** and **2b** alkylation product ratios for benzylic bromides **3a** and **3b** were not dependent on the reaction temperature in the range of 50 - 90°C (Table 1). When pure phenethyl derivatives **9a** and **9b** were treated with **2a** and **2b**, respectively, under the reaction conditions of alkylation, *i.e.*, heating in DMF for 24 h at 100°C in the presence of potassium carbonate, no migration of the substituent form N3 to N9 was observed. Therefore, the temperature dependent selectivity of the alkylation cannot be attributed to a reversible alkylation [15].

Debenzylation of **9b**, **10b** and **12b** by hydrogenolysis with 10% Pd on charcoal proceeded smoothly along with the reduction of bromine in the position 8 but **11b** was cleaved to adenine at the same reaction conditions. Therefore, allyl-protected derivatives **9a – 12a** were used preferentially to prepare the target compounds.

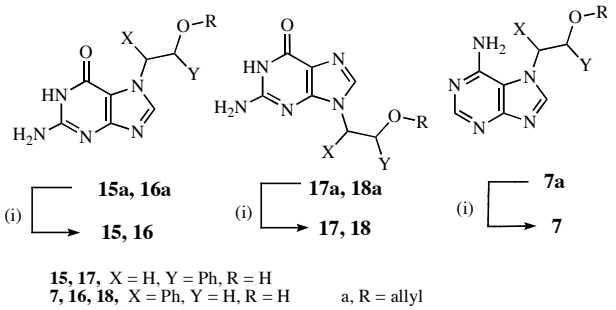
Allylic groups in bromoadenines **9a – 12a** were resistant to deallylation procedures such as cleavage with $NaBH_4/I_2$ [16], SmI_2 [17], DIBAL with [$NiCl_2$(dppp)] as a catalyst [18] and $PhSiH_3$ with $Pd(PPh_3)_4$ as a catalyst [19]. Catalytic deallylation using $Pd(PPh_3)_4$, $ZnCl_2$ and poly(methyl)hydrosiloxane (PMHS) [20] which we successfully applied previously to similar allyl-protected guanine derivatives [10,11], gave poor yields (10 % at most) with bromoadenines **9a** – **12a**. However, when $ZnCl_2$ was replaced by TsOH as a stronger acid, deallylation proceeded smoothly along with reduction of bromine in the position 8 (Scheme 3). Because isomeric N3- and N9-bromoadenines were more difficult to separate than the final deprotected product, mixtures of **9** and **10** or **11** and **12** were used as a starting material in most deprotection experiments. Final products were then separated by repeated column chromatography. So, pure 3-(2-hydroxy-2-phenylethyl)adenine (**13**) and 9-(2-4hydroxy-2-phenylethyl)adenine (**5**) were obtained by deallylation of a 3:1 mixture of **9a** and **10a** followed by separation of products. Similarly, deallylation of a 2:1 mixture of **11a** and **12a** yielded 3-(2-hydroxy-1-phenylethyl)adenine (**14**) and 9-(2-hydroxy-1-phenylethyl)adenine (**6**). This new deallylation procedure was also successfully applied to deprotection of N7-adenine derivative **7a** as well as of a series of guanine derivatives, namely, 7-(2-allyloxy-2-phenylethyl)guanine (**15a**), 7-(2-allyloxy-1-phenylethyl)guanine (**16a**), 9-(2-allyloxy-2-phenylethyl)guanine (**17a**) and 9-(2-allyloxy-1-phenylethyl)guanine (**18a**) (Scheme 4). The yields of deallylation are listed in the Table 2.

Scheme 3



(i) $Pd(PPh_3)_4$, TsOH, PMHS, DMF; (ii) H_2/Pd-C, MeOH

Scheme 4



(i) $Pd(PPh_2)_4$, TsOH, PMHS, DMF

For guanine derivatives they ranged from 74 - 96 % and were slightly better than those previously reported (68-90%) [11] with the catalytic system using $ZnCl_2$ as an acid.

Table 2

Yields of Deallylation

Starting Material	Product(s)	Yield [%]
9a	**13**	55
11a	**14**	45
9a + 10a	**13 + 5**	60
11a + 12a	**14 + 15**	59
7a	**7**	60
16a	**16**	96
17a	**17**	74
18a	**18**	90
19a	**19**	76

In the palladium complex catalyzed deallylation, coordination of the allylic π-electrons to palladium is essential to enable subsequent reductive cleavage of the allyl group. Basic nitrogen atoms in purines may coordinate to the transition metal and compete with the allylic π-electrons. Addition of an acid to the catalytic system seems to be necessary to prevent coordination of purine nitrogens to palladium. In the case of guanine derivatives $ZnCl_2$ was sufficient to neutralize the purine moiety, whereas adenine derivatives required TsOH as a stronger acid to achieve the same effect.

Table 3

^{13}C-NMR Chemical Shifts in ppm of Adenine Derivatives. Spectra were measured in $CDCl_3$ or [a]DMSO-d_6.

Compound	C2	C4	C5	C6	C8	CH_2	CH	aromatic CH and C	$CH=CH_2$ (allyl)
5[a]	152.3	149.6	118.5	155.9	141.3	50.3	70.7	125.9, 127.5, 128.2, 142.4	---
6a	152.4	149.6	118.7	156.0	139.7	69.9, 71.0	57.6	127.0, 128.0, 128.6, 138.0	116.8, 134.7
6b	153.1	150.5	119.7	155.1	140.0	70.6, 73.6	58.2	127.3, 127.9, 128.1, 128.6, 129.0, 129.3, 136.9, 137.3	---
7	152.3	151.8	111.7	160.2	144.8	63.3	62.2	127.1, 128.5, 129.2, 138.6	---
7a	152.2	151.3	111.0	159.8	144.2	70.5, 71.0	59.3	126.5, 128.2, 128.8, 137.8	117.0, 134.5
7b	152.6	151.7	112.6	160.1	145.1	71.2, 73.9	61.5	126.9, 128.0, 128.5, 128.7, 129.7, 129.8, 135.5, 136,2	---
9a	143.8	150.7	122.2	153.2	141.7	55.8, 70.0	78.0	126.9, 129.0, 129.1, 137.6	117.5, 133.8
9b[a]	145.1	150.4	122.0	154.3	140.0	55.0, 70.5	78.0	127.5, 127.7, 128.1, 128.7, 129.3, 129.5, 138.3, 138.7	---
10a	153.2	151.7	119.9	154.2	128.4	50.4, 69.9	78.7	126.8, 128.8, 128.9, 138.3	116.9, 134.1
10b	153.1	151.6	119.9	154.1	128.4	50.5, 70.7	77.8	127.0, 127.6, 127.7, 128.3, 128.8, 129.0137.5, 138.2	---
11a	143.9	151.3	122.3	152.8	141.5	68.9, 72.7	62.1	128.5, 129.3, 129.4, 135.4	118.3, 133.7
11b	142.8	151.3	122.3	152.8	141.8	68.8, 73.8	62.1	128.0, 128.5, 128.7, 129.3, 129.4, 135.2, 137.1	---
12a	152.7	151.8	120.3	154.6	128.4	69.2, 72.2	62.4	127.8, 128.7, 129.0, 136.1	117.6, 134.2
12b	152.7	151.9	120.3	154.3	128.7	69.0, 73.2	62.5	127.8, 127.85, 127.9, 128.5, 128.7, 129.0, 136.1, 137.7	---
13[a]	143.9	149.7	120.3	155.0	152.3	56.4	69.5	125.9, 127.5, 128.3, 142.1	---
14[a]	143.2	150.4	121.1	155.3	152.7	61.2	65.9	128.1, 128.7, 129.1, 137.5	---
15[a]	153.1	150.5	119.5	156.7	139.9	63.1	61.1	127.8, 128.7, 129.4, 138.9	---

The position of the alkyl at the purine moiety was determined unequivocally by 2D NMR experiments. Parameters were set to show interactions $J \approx 7$ Hz corresponding to three-bond C-H coupling constants on the heteroaromatic ring. Protons of NCH_2 or NCH-CH_2 showed cross-peaks with corresponding carbons of the purine rings. To assign 2-*H* and 8-*H* protons in the adenine derivatives unequivocally, their single bond C-H correlation in HMQC was not sufficient because corresponding carbon signals were very close. In HMBC, 2-*H* protons showed cross-peaks with both C-4 and C-6 and, similarly, 8-*H* protons correlated with C-4 and C-5. In all cases the resonances of 2-*H* was found at lower field values (8.14 - 8.56 ppm) than those of 8-*H* (7.70 - 8.10 ppm). This is in agreement with an earlier observation of Kjellberg and Johansson [21].

EXPERIMENTAL

Column chromatography was performed on silica gel 60 purchased from Fluka, particle size 0.063-0.200 mm. For thin-layer chromatography Merck Silica gel 60 F_{254} plates were used. Dimethylformamide (DMF) was dried by vacuum distillation from phosphorus pentoxide and stored over molecular sieves. Other chemicals obtained from commercial sources were of analytical or synthetic grade and were used as received. ^{1}H and ^{13}C NMR spectra were recorded with Bruker Avance DRX500 (500 MHz for ^{1}H) or with Varian Mercury 300 (300 MHz for ^{1}H) Fourier transform NMR spectrometer. Mass spectra were measured on a triple quadrupole HPLC-MS system Varian 1200 equipped with electrospray ionization (ESI) in the positive ion mode.

Starting Materials. 8-Bromoadenine (**8**) was prepared by bromination of adenine in aqueous solution [22]. Alkylating reagents, *i.e.*, 2-bromo-1-phenylethanol (**2**) [23], allyl(2-bromo-1-phenylethyl) ether (**2a**) [11], allyl(2-bromo-2-phenylethyl) ether (**3a**) [11], benzyl(2-bromo-1-phenylethyl) ether (**2b**) [11], benzyl(2-bromo-2-phenylethyl) ether (**3b**) [24] and 2-benzyl-oxy-2-phenylethyl triflate (**4**) [12] were prepared according to published procedures.

Alkylation of adenine with 2-bromo-1-phenylethanol (2). A mixture of 200 mg (1.48 mmol) of adenine, 205 mg (1.28 mmol) of potassium carbonate, 300 mg (1.14 mmol) of 18-crown-6 and 607 mg (3 mmol) of bromohydrin **2** in 20 mL of DMF was heated to 125°C for 20h under dry nitrogen. Three products R_f = 0.49, 0.39 and 0.34 were detected by TLC (CH_2Cl_2-MeOH 6:1). The reaction mixture was evaporated to dryness and separated by repeated column chromatography on silica gel.

9-(2-Hydroxy-2-phenylethyl)adenine (5). This compound was obtained by alkylation of adenine with **2** (109 mg, 29%) and also by deallylation of a 3:1 mixture of **9a** and **10a** (150 mg, 0.4 mmol). Repeated column chromatography yielded 22 mg (87 %)

of a white powder, mp > 250°C; R_f (CH_2Cl_2-MeOH 6:1) = 0.49; ^{1}H NMR (DMSO-d_6): δ 4.26 (m, 2H, C*H*$_2$N); 4.98 (m, 1H, PhC*H*); 5.79 (d, 1H, J = 4.6 Hz, O*H*); 7.16 (br, 2H, N*H*$_2$); 7.27 (m, 1H, *Ph*); 7,35 (m, 4H, *Ph*); 7.98 (s, 1H, 8-*H*); 8.14 (s, 1H, 2-*H*); HMBC: Cross-peak of NC*H*$_2$ at δ = 4.26 ppm with *C*-8 at 142.4 ppm and *C*-4 at 149.6 ppm; ESI-MS: m/z = 256 $[M+H]^+$, 278 $[M+Na]^+$. *Anal.* Calcd. for $C_{13}H_{13}N_5O$: C, 61.2; H, 5.1; N, 27.4. Found: C, 60.9; H, 5.2; N, 27.3.

Alkylation of Adenine with Allyl or Benzyl (2-Bromo-2-phenylethyl) Ether (3a or 3b). Adenine (200 mg, 1.49 mmol), NaH (36 mg, 1.4 mmol) and alkylating reagent **3a** or **3b** (2.2 mmol) were added to 15 mL of DMF. The reaction mixture was stirred under a dry nitrogen atmosphere for two weeks at room temperature, then it was diluted with 15 mL of chloroform, the undissolved portion was filtered off, and the filtrate was evaporated to dryness *in vacuo* and separated by repeated column chromatography on silica gel using 10:1 and 6:1 $CHCl_3$-MeOH as an eluent.

9-(2-Allyloxy-1-phenylethyl)adenine (6a). A white powder obtained by alkylation of adenine with **3a**, crystallized from chloroform – cyclohexane, 87 mg (20%), mp 158-160°C, R_f ($CHCl_3$ - MeOH 6:1) = 0.50; ^{1}H-NMR ($CDCl_3$): δ 4.00 (d, 2H, J = 5.5 Hz, C*H*$_2$O); 4.05 (dd, 1H, J = 10.5 and 4.4 Hz, C*H*$_2$CHN), 4.13 (dd, 1H, J = 10.5 and 6.9 Hz, C*H*$_2$CHN); 5.16 (d, 1H, J = 9.1 Hz, C*H*$_2$=CH); 5. 18 (d, 1H, J = 14.6 Hz, C*H*$_2$=CH); 5.94 (dd, 1H, J = 6.9 and 4.4 Hz, NC*H*); 6.2 (br, 2H, N*H*$_2$); 7.30 (m, 5H, *Ph*); 8.00 (s, 1H, 8-*H*), 8.33 (s, 1H, 2-*H*); HMBC: Cross-peaks of NC*H* at δ = 5.94 ppm with *C*-8 at 139.7 ppm and *C*-4 at 152.4 ppm were detected. ESI-MS: m/z 296 $[M+H]^+$, 318 $[M+Na]^+$ and 334 $[M+K]^+$. *Anal.* Calcd. for $C_{16}H_{17}N_5O \times 1/4$ H_2O: calc. C, 64.1; H, 5.9; N, 23.7; Found: C, 64.2; H, 5.8; N, 23.3.

7-(2-Allyloxy-1-phenylethyl)adenine (**7a**). A minor product of the alkylation of adenine with **3a** obtained by repeated column chromatography and crystallization from chloroform as a white powder, 37 mg (8 %), mp 189-191°C, R_f ($CHCl_3$ - MeOH 6:1) = 0.39; ^{1}H-NMR ($CDCl_3$): δ 4.04 (m, 2H, C*H*$_2$O); 4.19 (dd, 1H, *J* = 10.5 and 8 Hz, C*H*$_2$CHN), 4.21 (dd, 1H, *J* = 10.5 and 3.9 Hz, C*H*$_2$CHN); 5.19 (m, 2H, C*H*$_2$=CH); 5.82 (dd, 1H, *J* = 7.7 and 3.9 Hz, NC*H*); 7.20 (m, 3H, *Ph*); 7.40 (m, 2H, *Ph*); 8.02 (s, 1H, 8-*H*); 8.43 (s, 1H, 2-*H*); HMBC: Cross-peaks of NC*H* at δ = 5.82 ppm with *C*-8 at 144.2 ppm and *C*-5 at 111.0 ppm were detected. ESI-MS: m/z 296 $[M+H]^+$, 318 $[M+Na]^+$ and 334 $[M+K]^+$; *Anal.* Calcd. for $C_{16}H_{17}N_5O$: C, 65.1; H, 5.8; N 23.7; Found: C, 64.7; H, 5.7; N 23.3.

9-(2-Benzyloxy-1-phenylethyl)adenine (7b). This compound was obtained by alkylation of adenine with **3b**. Repeated column chromatography followed by crystallization from chloroform - cyclohexane yielded 94 mg (18%) of a white powder, mp 197-194°C, R_f ($CHCl_3$ - MeOH 8:1) = 0.71; ^{1}H-NMR ($CDCl_3$): δ 4.09 (dd, 1H, J = 10.4 and 4.4 Hz, NCHC*H*$_2$); 4.35 (dd, 1H, J = 10.4 and 6.9 Hz, NCHC*H*$_2$); 4.53 and 4.58 (d, 1+1H, PhC*H*$_2$O); 5.59 (br, 2H, N*H*$_2$); 5.93 (dd, 1H, J = 6.9 and 4.4 Hz, C*H*N); 7.19 (m, 2H, *Ph*); 7.30 (m, 8H, *Ph*); 7.98 (s, 1H, 8-*H*); 8.32 (s, 1H, 2-*H*); ESI-MS: m/z 346 $[M+H]^+$, 368 $[M+Na]^+$; *Anal.* Calcd. for $C_{20}H_{19}N_5O \times 1/4$ H_2O: C, 68.7; H, 5.6; N, 20.0; Found: C, 68.5; H, 5.8; N, 20.3.

7-(2-Benzyloxy-1-phenylethyl)adenine (7b). A minor product of the alkylation of adenine with **3b**. Repeated column chromatography and crystallization from chloroform - cyclohexane yielded 59 mg (11.5 %) of a white powder, mp 211-215°C, R_f ($CHCl_3$ - MeOH 8:1) = 0.61; ^{1}H-NMR ($CDCl_3$): δ 4.19 (dd, 1H, J = 10.5 and 8.2 Hz, NCHC*H*$_2$); 4.24 (dd, 1H, J = 10.5 and 3.6 Hz, NCHC*H*$_2$); 4.54 and 4.59 (d, 1+1H, J = 11.8 Hz, PhC*H*$_2$O); 5.02 (br, 2H, N*H*$_2$); 5.82 (dd, 1H, J = 8.2 and 3.6 Hz, C*H*N); 7.19 (m, 2H, *Ph*); 7.30 (m, 3H, *Ph*); 7. 41 (m, 5H, *Ph*); 8.03 (s, 1H, 8-*H*); 8.46 (s, 1H, 2-*H*); ESI-MS: m/z 346 $[M+H]^+$, 368 $[M+Na]^+$; *Anal.* Calcd. for $C_{20}H_{19}N_5O$: C, 69.5; H, 5.5; N, 20.3; Found: C, 69.2; H, 5.8; N, 20.3.

General Procedure for Alkylation of 8-Bromoadenine (8). A mixture of 250 mg (1.17 mmol) of **8**, 242 mg (1.75 mmol) of potassium carbonate, 2.34 - 7.02 mmol of alkylating agent **2a**, **2b**, **3a** or **3b** and 10 mL of DMF was stirred under a nitrogen atmosphere at 50 - 100°C and the course of reaction was followed by TLC (Silica gel 60 F254, Merck, $CHCl_3$-MeOH 8:1). After 4 - 72 h, when the spot of **8** at R_f = 0.16 disappeared, the solvent was distilled off *in vacuo*, the residue was re-suspended in 25 mL of dichloromethane - ethyl acetate 2:1, poured onto a bed of 25 g silica gel (for flash chromatography) and eluted subsequently with 100 mL of dichloromethane, 80 mL of ethyl acetate and 80 mL of ethanol. Crude products, bromoadenines **9a,b** - **12a,b**, 2 for each alkylating reagent used, were eluted in the ethyl acetate fraction. They were analyzed by ^{1}H-NMR to determine the ratio of N3:N9 substituted product by integration of corresponding 2-*H* signals and used directly in further reaction steps. Pure samples of **9a,b** - **12a,b** were obtained by column chromatography on silica gel followed by crystallization or by semi-preparative HPLC.

When triflate **4** was used as an alkylation reagent instead of protected bromohydrins, full conversion of bromoadenine **8** was achieved in 4 h at room temperature.

8-Bromo-3-(2-allyloxy-2-phenylethyl)adenine (9a) and 8-Bromo-9-(2-allyloxy-2-phenylethyl)adenine (10a). These compounds were obtained by alkylation of **8** by **2a**. Crystallization of 350 mg of the crude product containing 7:2 mixture of **9a** and **10a** from dichloromethane - petroleum ether afforded 32 mg (12%) of **9a**. Remaining mixture of the two isomers was separated by semi-preparative HPLC on a 250 x 8 mm Watrex C18 column (50 - 80 % MeOH, 27 min; 80% MeOH for additional 5 min) affording analytical samples of pure **9a** and **10a**.

9a: White powder, mp 188-189°C; R_f ($CHCl_3$-MeOH 10:1) = 044.; ^{1}H-NMR ($CDCl_3$): δ 3.64 (dd, 1H, J = 12.6 and 5.9 Hz, OC*H*$_2$); 3.90 (dd, 1H, J = 12.6 and 5.00Hz, OC*H*$_2$); 4.22 (dd, 1H, J = 9.5Hz and 13.6Hz, NC*H*$_2$); 4.67 (dd, 1H J = 3.1Hz and 13.63Hz, NC*H*$_2$); 4.85 (dd, 1H, J = 9.2 and 3.1Hz, PhC*H*); 5.05 (m, 2H CH=C*H*$_2$); 5.63 (m, 1H, CH$_2$=C*H*); 6.2-6.6 (br, 2H, N*H*$_2$); 7.25-7.38 (m, 5H, *Ph*); 7.99 (s, 1H, 2-*H*); HMBC: Cross-peaks of NC*H*$_2$ (δ = 4.22 and 4.67 ppm) with the signals of *C*-2 at 143.8 ppm and *C*-4 at 150.7 ppm as well as the cross-peak of 2-*H* (δ = 7.99 ppm) with the signal of *C*H$_2$N at 55.8 ppm; ESI-MS (m/z): 374 and 376 $[M+H]^+$; *Anal.* Calcd. for $C_{16}H_{16}N_5OBr.1/3$ H_2O: C, 50.5; H, 4.4; N, 18.4; Found: C, 50.2; H, 4.0; N, 18.4.

10a: White powder, mp 151-153°C; R_f ($CHCl_3$-MeOH 10:1) = 0.48; ^{1}H-NMR ($CDCl_3$): δ 3.65 (m, 1H, OC*H*$_2$); 3.92 (m, 1H, OC*H*$_2$); 4.28 (dd, 1H, J = 14.1 and 4.7Hz, NC*H*$_2$); 4.49 (dd, 1H, J = 14.1 and 8.8 Hz, NC*H*$_2$); 4.87 (dd, 1H, J = 8.8 and 4.7Hz, PhC*H*); 5.04 (m, 2H, CH=C*H*$_2$); 5,61 (m, 1H, CH$_2$=C*H*); 5.73 (br, 2H, N*H*$_2$); 7.27-7.40 (m, 5H, *Ph*); 8.34 (s, 1H, 2-*H*); HMBC: Cross-peaks of N-C*H*$_2$ (δ = 4.28 and 4.49 ppm) with the signals of *C*-8 at 128.4 ppm and *C*-4 at 151.6 ppm; ESI-MS: m/z 374 and 376 $[M+H]^+$; *Anal.* Calcd. for $C_{16}H_{16}N_5OBr.1/3$ H_2O: C, 50.2; H, 4.2; N, 18.4; Found: C, 50.5; H, 4.4; N, 18.4.

8-Bromo-3-(2-benzyloxy-2-phenylethyl)adenine (9b) and 8-Bromo-9-(2-benzyloxy-2-phenylethyl)adenine (10b). These compounds were obtained by alkylation of **8** either with **2b** or with triflate **4**. Pure analytical samples of **9b** and **10b** were obtained by semi-preparative HPLC on a 250 × 8 mm Watrex C18 column using a methanol - water gradient (63 - 90 % MeOH, 25 min; 90% MeOH for additional 10 min).

9b: White powder, mp 226-227°C; R_f ($CHCl_3$-Me_2CO-MeOH 15:1:1) = 0.47; ^{1}H-NMR (DMSO-d_6): δ 4.14 (d, 1H, J = 12.2 Hz, CH_2O); 4.37 (d, 1H, J = 12.2 Hz, CH_2O); 4.43 (dd, 1H, J = 13.6 and 3.8 Hz, CH_2N); 4.50 (dd, 1H, J = 13.6 and 9.2 Hz, CH_2N); 4.92 (dd, 1H, J = 9.2 and 3.7 Hz, C*H*O); 6.97 (m, 2H, *Ph*); 7.19 (m, 3H, *Ph*); 7.40 (m, 1H, *Ph*); 7.46 (m, 4H, *Ph*); 8.05 (br, 1H, N*H*) 8.22 (br, 1H, N*H*); 8.26 (s, 1H, 2-*H*); HMBC: Cross-peaks of NCH_2 (δ = 4.43 and 4.5 ppm) with the signals of *C*-2 at 145 ppm, *C*-4 at 150 ppm and aromatic *C* at 138.7 ppm; ESI-MS: m/z 424 and 426 $[M+H]^+$, 446 and 448 $[M+Na]^+$, 346 $[M-Br]^+$; *Anal*. Calcd. for $C_{20}H_{18}N_5OBr.1/4H_2O$: C, 56.0; H, 4.2; N, 16.4; Found C, 56.4; H, 3.8; N, 16.5.

10b: White powder, mp 146-147°C; R_f ($CHCl_3$-Me_2CO-MeOH 15:1:1) = 0.43; ^{1}H-NMR ($CDCl_3$): δ 4.20 (d, 1H, J = 12 Hz, CH_2O) 4.50 (d, 1H, J = 12 Hz, CH_2O); 4.25 (dd, 1H, J = 14.2 and 4.1 Hz, CH_2N); 4.50 (dd, 1H, J = 14.2 and 9.6 Hz, CH_2N); 4.87 (dd, J = 9.7 and 4.1 Hz, C*H*O); 5.6 (br, 2H, NH_2); 6.92 (m, 2H, *Ph*); 7.14 (m, 3H, *Ph*); 7.42 (m, 5H, *Ph*); 8.27 (s, 1H, 2-*H*); HMBC: Cross-peaks of NCH_2 (δ = 4.25 and 4.50 ppm) with the signals of *C*-8 at 128.4 ppm and *C*-4 at 151.6 ppm; ESI-MS: m/z 424 and 426 $[M+H]^+$, 446 and 448 $[M+Na]^+$, 346 $[M-Br]^+$; *Anal*. Calcd. for $C_{20}H_{18}N_5OBr.1/4H_2O$: C, 56.0; H, 4.2; N, 16.4; Found: C, 56.2; H, 4.2; N, 16.3.

8-Bromo-3-(2-allyloxy-1-phenylethyl)adenine (11a) and 8-Bromo-9-(2-allyloxy-1-phenylethyl)adenine (12a). These compounds were obtained by alkylation of **8** with **3a**. Separation by semi-preparative HPLC on a 250 × 8 mm Watrex C18 column using a MeOH - H_2O gradient (50 - 80 % MeOH, 27 min; 80% MeOH for additional 5 min) afforded analytical samples of pure compounds **11a** and **12a**.

11a: White powder, mp 158-160°C; R_f ($CHCl_3$-MeOH 8:1) = 0.53; ^{1}H-NMR ($CDCl_3$): δ 4.00 (d, 2H, J = 5.6 Hz, CH_2=CH-CH_2); 4.10 (dd, 1H J = 10.9 and 4.4 Hz, NCHCH_2O); 4.40 (dd, 1H, J = 10.9 and 4.7 Hz, NCHCH_2O); 5.20 (m, 2H, CH_2=CH); 5.80 (m, 1H, CH_2=C*H*); 6.29 (t, 1H, J = 4.3 Hz, NC*H*). HMBC: Cross-peaks of NC*H* (δ = 6.29 ppm) with the signals of *C*-2 at 144 ppm and *C*-4 at 151 ppm; ESI-MS: m/z 374 and 376 $[M+H]^+$, 396 and 398 $[M+Na]^+$, 412 and 414 $[M+K]^+$; *Anal*. Calcd. for $C_{16}H_{16}N_5OBr.H_2O$: C, 49.0; H, 4.6; N, 17.9; Found C, 48.9; H, 4.2; N, 17.7.

12a: White powder, mp > 250°C; R_f ($CHCl_3$-MeOH 8:1) = 0.53; ^{1}H-NMR ($CDCl_3$): δ 4.00 (d, 2H, J = 5.9 Hz, CH_2=CH-CH_2); 4.15 (dd, 1H, J = 10.2 and 5.1 Hz, NCHCH_2O); 5.04 (t, 1H, J = 10.2 Hz, NCHCH_2O); 5.11 (d, 1H, J = 10.9 Hz, CH_2=CH); 5.16 (dd, 1H, J = 18 and 1.5 Hz, CH_2=CH); 5.76 (m, 1H, CH_2=C*H*); 5.87 (dd, 1H, J = 10 and 5 Hz, NC*H*); ESI-MS: m/z 374 and 376 $[M+H]^+$, 396 and 398 $[M+Na]^+$, 412 and 414 $[M+K]^+$; Cross-peaks of NC*H* (δ = 5.87 ppm) with the signal of *C*-4 at 152 ppm and with aromatic *C*H overlapping *C*-8 at 128 ppm were observed. *Anal*. Calcd. for $C_{16}H_{16}N_5OBr$: C, 51.4; H, 4.3; N, 18.7; Found C, 51.5, H, 4.6; N, 18.3.

8-Bromo-3-(2-benzyloxy-1-phenylethyl)adenine (11b) and 8-Bromo-9-(2-benzyloxy-1-phenylethyl)adenine (12b). These compounds were obtained by alkylation of **8** with **3b**. Repeated column chromatography of the crude product on silica gel (32 g) with $CHCl_3$ - Me_2CO - MeOH 15:1:1 as an eluent yielded 40 mg (8%) of **11b** and 25 mg (5 %) of **12b**.

11b: White solid, mp > 250°C; R_f ($CHCl_3$-Me_2CO-MeOH 15:1:1) = 0.21; ^{1}H-NMR ($CDCl_3$): δ 4.16 (dd, 1H, J = 10.8 and 4.3 Hz, CHCH_2O); 4.40 (dd, 1H, J = 10.8 and 4.6 Hz, CHCH_2O); 4.53 (s, 2H, PhCH_2O); 6.29 (t, 1H, J = 4.4 Hz, NC*H*); 7.19 (m, 2H, *Ph*); 7.30 (m, 3H, *Ph*); 7.38 (m, 3H, *Ph*); 7.42 (m, 2H, *Ph*); 8.00 (s, 1H, 2-*H*); HMBC: Cross-peaks of NC*H* (δ = 6.29 ppm) with the signals of *C*-2 at 143 ppm and *C*-4 at 151 ppm; ESI-MS: m/z 424 and 426 $[M+H]^+$, 446 and 448 $[M+Na]^+$, 462 and 464 $[M+K]^+$; *Anal*. Calcd. for $C_{20}H_{18}N_5OBr.1/4H_20$: C, 56.0; H, 4.2; N, 16.4; Found: C, 56.2; H, 4.5; N, 16.6.

12b: White solid, mp > 250°C; R_f ($CHCl_3$-Me_2CO-MeOH 15:1:1) = 0.15; ^{1}H-NMR ($CDCl_3$): δ 4.15 (dd, 1H, J = 10 and 5 Hz, CHCH_2O); 5.09 (t, 1H, J = 5.0 Hz, CHCH_2O); 4.51 (d, 1H, J = 12.1 Hz, PhCH_2O); 4.55 (d, 1H, J = 12.1 Hz, PhCH_2O); 5.7 (br s, 2H, NH_2); 5.89 (t, 1H, J = 5 Hz, NC*H*); 7.13 (m, 2H, *Ph*); 7.25 (m, 4H, *Ph*); 7.32 (m, 4H, *Ph*); 7.47 (m, 2H, *Ph*); 8.26 (s, 1H, 2-*H*); HMBC: Cross-peaks of NC*H* (δ = 5.89 ppm) with the signals of *C*-8 at 128.7 ppm and *C*-4 at 151.9 ppm; ESI-MS: m/z 424 and 426 $[M+H]^+$, 446 and 448 $[M+Na]^+$, 462 and 464 $[M+K]^+$; *Anal*. Calcd. for $C_{20}H_{18}N_5OBr.1/4H_20$: C, 56.0; H, 4.2; N, 16.4; Found C, 56.0; H, 4.1; N, 16.2.

General Procedure for Debenzylation. Palladium catalyst (750 mg, 10% Pd-C, Degusa type, wet, containing approx. 50% of water) was activated by heating to 100°C for 1 h *in vacuo*. Argon was introduced into the reaction flask with the activated catalyst, 400 mg (6.35 mmol) of ammonium formate and a solution of 150 mg (0.353 mmol) of a bezyl protected compound (**9b**, **10b**, **12b**, a mixture **9b** and **10b** or **11b** and **12b**) in absolute MeOH (20 mL) was added. The reaction mixture was heated in an oil bath to 60°C under an argon atmosphere. After 1h another portion of 400 mg (6.35 mmol) of ammonium formate was added and the reaction was continued for an additional 1h. The catalyst was filtered off and washed with aqueous MeOH. The solvents were evaporated *in vacuo* to yield crude products.

General Procedure for Deallylation. To a stirred solution of 150 mg (0.4 mmol) of an allyl-protected adenine derivative (pure **9a**, **11a** or a mixture of either **9a** + **11a** or **10a** + **12a**) in DMF (10 mL) under an argon atmosphere, 150 mg (0.8 mmol) of TsOH monohydrate, 14 mg (0.012 mmol) of $Pd(PPh_3)_4$ and 100 μL of PMHS was added and the reaction mixture was heated to 90°C. After 3 and 6 h additional portions of PMHS were added (2 × 100 μL). When the reaction was complete (after 9 - 20 h), the solvent was evaporated *in vacuo* and the products were isolated by repeated column chromatography. For deallylation of guanine derivatives **15a** – **18a** (125 mg; 0.4 mmol) lower amounts of TsOH (75 mg; 0.4 mmol) and PMHS (100 μL) were used. Full conversion of the starting material was achieved in 3 h. Resulting alkylguanines were purified by column chromatography on silica gel followed by anion exchange on Dowex 1 to remove coeluting TsOH.

3-(2-Hydroxy-2-phenylethyl)adenine (13). Deallylation of **9a** followed by crystallization from ethanol yielded 56 mg (55 %) of **13**. Deallylation of a 3:1 mixture of **9a** and **10a** followed by repeated column chromatography yielded 25 mg (24%) of **13** (the yield corrected to the content of **9a** in the starting material). Also obtained by debenzylation of 100 mg of **9b** and purification by column chromatography ($CHCl_3$-MeOH 3:1) as a white powder, 33 mg (55 %), mp > 250°C; R_f ($CHCl_3$-MeOH 8:1) = 0.15; ^{1}H-NMR (DMSO-d_6): δ 4.23 (dd, 1H, J = 13.5 and

9.4 Hz, CH_2); 4.48 (dd, 1H, J = 13.5 and 3.4 Hz, CH_2); 5.1 (m, 1H, C*H*OH): 5.9 (br, 1H, O*H*); 7.23-7.45 (m, 5H, *Ph*); 8.18 (s, 1H, 2-*H*); 7.78 (s, 1H, 8-*H*); HMQC: Cross-peaks of 2-*H* at δ = 8.18 ppm with *C*-2 at 144 ppm and 8-*H* at δ = 7.78 ppm with *C*-8 at 152 ppm; HMBC: Cross-peaks of NCH_2 (δ = 4.23 and 4.48 ppm) with *C*-2 at 144 ppm and with *C*-4 at 149.7 ppm; ESI-MS: m/z 256 $[M+H]^+$, 278 $[M+Na]^+$; *Anal*. Calcd. for $C_{13}H_{13}N_5O$: C, 61.2; H, 5.1; N, 27.4; Found C, 60.8; H, 5.1; N, 27.3.

3-(2-Hydroxy-1-phenylethyl)adenine (14). This compound was obtained by deallylation of **12a** (45%) and of a 2:1 mixture of **11a** and **12a** (150mg, 0.4 mmol). Repeated column chromatography followed by crystallization from hot aqueous ethanol yielded 23 mg (33%) of **14** (the yield corrected to the content of **11a** in the starting material) as a white powder, mp > 250°C; R_f ($CHCl_3$-MeOH 6:1) = 0.29; ^{1}H-NMR (DMSO-d_6): δ 4.12 (dd, 1H, J = 11.3 and 3.5 Hz, CH_2OH); 4.60 (m, 1H, CH_2OH); 5.92 (dd, 1H, J = 9.1 and 5 Hz, C*H*N); 5.45 (br, 1H, O*H*); 7.30 (m, 3H, *Ph*); 7.45 (dd, 2H, J = 8.0 and 1.5 Hz, *Ph*); 7.90 (br, 2H, NH_2); 7.70 (s, 1H, 8-*H*); 8.56 (s, 2-*H*); HMBC: Cross-peaks of C*H*N (δ = 5.92 ppm) with *C*-2 at 143.2 and with *C*-4 at 150.4; ESI-MS: m/z 256 $[M+H]^+$, 278 $[M+Na]^+$; *Anal*. Calcd. for $C_{13}H_{13}N_5O$: C, 61.2; H, 5.1; N. 27.4; Found: C, 60.9; H, 5.1; N, 27.3.

9-(2-Hydroxy-1-phenylethyl)adenine (6) [25]. This compound was obtained by deallylation of a 2:1 mixture (150 mg; 0.4 mmol) of **11a** and **12a**. Separation of the crude product by column chromatography yielded 19 mg (57%) of **15**. Alternatively, debenzylation of 150 mg (0.353 mmol) of a 1:1 mixture of **11a** and **12a** followed by separation on a silica gel column using $CHCl_3$ - MeOH 4:1 as an eluent yielded 15 mg (62%) of adenine and 28 mg (62%) of **15** as a white powder, mp > 250°C; R_f ($CHCl_3$-MeOH 8:1) = 0.32; ^{1}H-NMR (DMSO-d_6): δ 4.03 (m, 1H, CH_2OH); 4.39 (m, 1H, CH_2OH); 5.68 (dd, 1H, J = 8.9 and 4.9 Hz, CH_2N); 5.31 (t, 1H, J = 5.4 Hz, O*H*); 7.30 (m, 5H, *Ph*); 7.22 (br s, 2H, NH_2); 8.10 (s, 1H, 8-*H*); 8.41 (s, 1H, 2-*H*); ESI-MS: m/z 256 $[M+H]^+$, 278 $[M+Na]^+$; *Anal*. Calcd. for $C_{13}H_{13}N_5O.1/4H_2O$: C, 60.1; H, 5.2; N, 27.0; Found: C, 59.9; H, 5.1; N, 27.0.

9-(2-Hydroxy-1-phenylethyl)guanine (18). This compound was obtained by deallylation of **18a** (78 mg; 0.25 mmol). Reaction mixture was evaporated to dryness *in vacuo*, the residue was re-suspended in $CHCl_3$ - MeOH 3:1 and poured onto a silica gel bed (10 g). Elution with $CHCl_3$ - MeOH 3:1 yielded a product, which was contaminated with TsOH. Pure **18** was obtained by crystallization from MeOH - $CHCl_3$ (20 mg, 29%). Another portion, 30 mg of the product was obtained by separation on Dowex 1 anion exchange resin (3g). Total yield was 50 mg (74%) of a white powder, mp > 250°C, R_f ($CHCl_3$-MeOH) = 0.19; ^{1}H-NMR (DMSO-d_6): δ 3.98 (m, 1H, CH_2O); 4.41 (m, 1H, CH_2O); 5.25 (t, 1H, J = 5.4 Hz, O*H*); 5.44 (dd, 1H, J = 8.7 and 5.1 Hz, C*H*N); 6.37 (br s, 2H, NH_2); 7.28 (m, 5H, *Ph*); 7.94 (s, 1H, 8-*H*); ^{13}C-NMR (DMSO-d_6): 60.6 (*C*HN); 63.2 (*C*H_2O); 105.0 (*C*5); 127.5, 128.3 and 129.3 (Ar *C*H); 138.7 (*C*); 152.0 (*C*2); 154.4 (*C*4); 157.0 (*C*6); ESI-MS: m/z 272 $[M+H]^+$, 294 $[M+Na]^+$, 310 $[M+K]^+$; *Anal*. Calcd. for $C_{13}H_{13}N_5O_2$: C, 57.6; H, 4.8; N, 25.8; Found: C, 57.4; H, 4.9; N, 25.5.

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