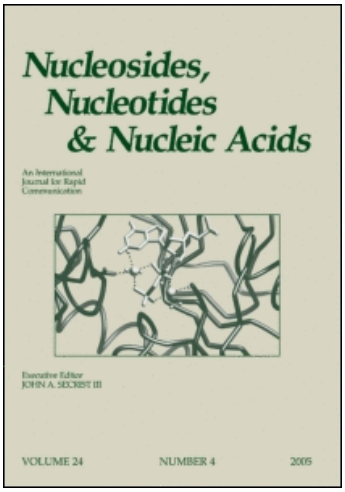

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UNEXPECTED DEHALOGENATION OF 3-BROMOPYRAZOLO[3,4-d]PYRIMIDINE NUCLEOSIDES DURING NUCLEOBASE-ANION GLYCOSYLATION

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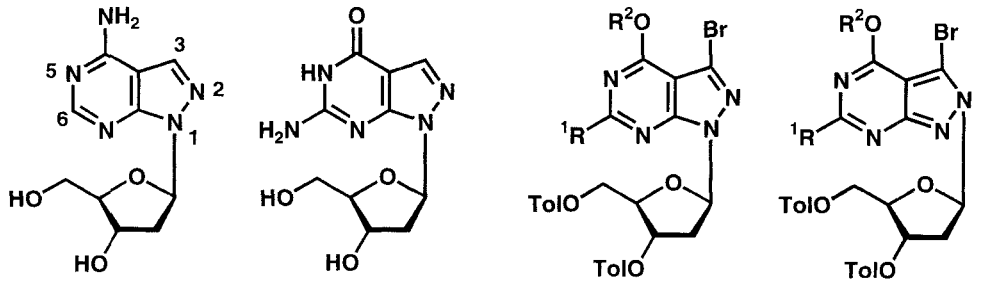
ABSTRACT: The anion-glycosylation (KOH, MeCN, TDA-1) of 3-bromopyrazolo[3,4-d]-pyrimidines **4a** and **4b** with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (**5**) furnishes the regioisomeric N^1-ß-D-2'-deoxyribonucleosides **6a** and **6b** together with the dehalogenated N^2-regioisomers **8a** and **8b**, stereoselectively. The dehalogenation takes place after the glycosylation and results from the sensitivity of the N-2 nucleosides toward aqueous base. An addition/elimination mechanism is suggested for the dehalogenation reaction.

Certain pyrazolo[3,4-d]pyrimidines exhibit strong therapeutic activity against various diseases.[1-2] Allopurinol is the drug of choice for the treatment of gout.[3] Also pyrazolo[3,4-d]pyrimidine ribonucleosides including those with 3-bromo or 3-iodo substituents develop biological action in particular antiparasitic activity and inhibitory action against adenosine kinases.[4-6] The 2'-deoxyribonucleosides, such as **1** or **2** have been incorporated in oligonucleotides chemically and enzymatically.[7,8]

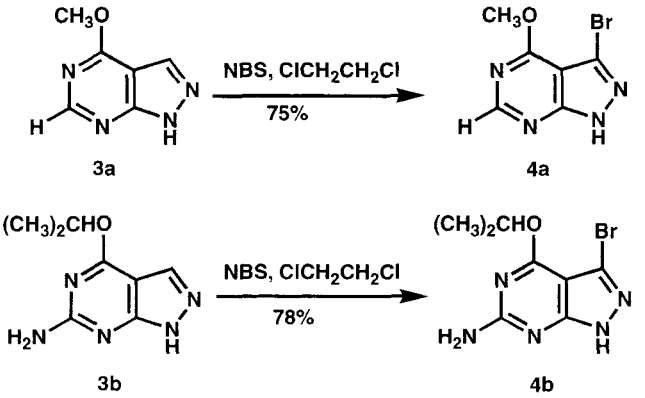
Recently, the stabilizing effect of pyrrolo[2,3-d]pyrimidines on the oligonucleotide duplex structure was reported.[9] In these cases the 5-membered ring of the base carries a halogen substituent. As the same favorable properties, e.g. stabilization of an oligonucleotide duplex, are expected for 3-halogenated pyrazolo[3,4-d]pyrimidines [2,10,11] the synthesis of corresponding 2'-deoxyribonucleosides was considered. As central intermediates the alkoxy derivatives **6a,b** were chosen. These intermediates should be amenable to conversion to nucleosides with various substituents at carbon-4.



1 2 6a,b 7a,b

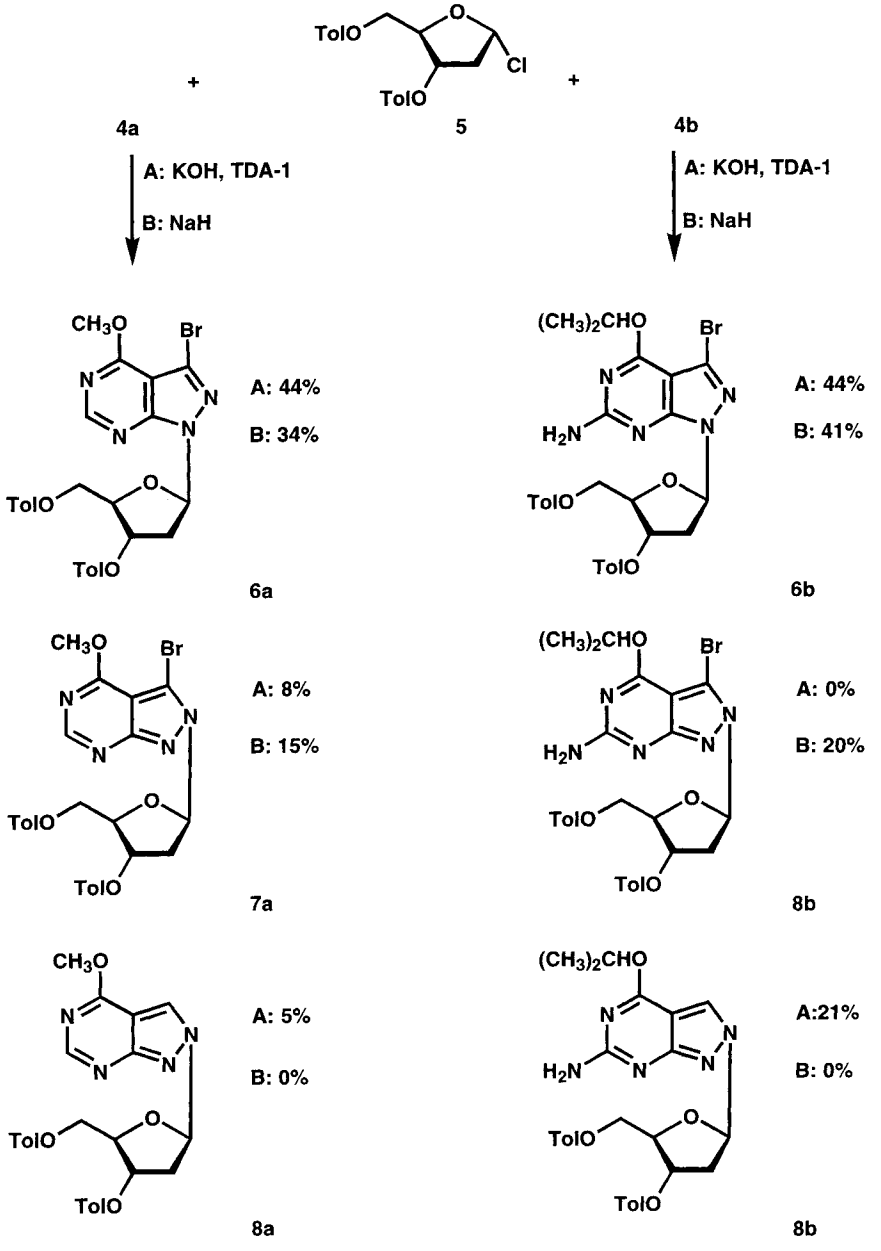
a: $^1R = H$; $^2R = CH_3$ b: $^1R = NH_2$; $^2R = CH(CH_3)_2$

The pyrazolo[3,4-d]pyrimidines **3a,b** which have been described before[12,13] served as starting materials. They were brominated with N-bromosuccinimide (NBS) in 1,2-dichloroethane to give the bromo compounds **4a,b** in 75% and 78% yield, respectively.



These compounds were employed in the stereoselective nucleobase-anion glycosylation using 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (**5**)[14].

The reaction was performed at room temperature in MeCN with powdered KOH (containing 15% of water) as base and TDA-1 (tris[2-(2-methoxyethoxy)ethyl]amine) as catalyst.[15] The glycosylation products were purified by flash chromatography. Three zones were separated in the case of the glycosylation reaction performed on compound **4a**. The first zone contains the brominated N^1-isomer **6a** (44%). The second zone furnishes the brominated N^2-isomer **7a** (8%) whereas the third zone yielded **8a** (5%



yield) which did not contain bromine. According to analytical and spectroscopic data structure **8a** was established and the compound was found to be identical to a protected nucleoside, which has been synthesized before.[12] The same glycosylation reaction was performed on compound **4b** furnishing only two reaction products. They were also separated chromatographically. The compound of the fast migrating zone was characterized as the bromo nucleoside **6b** (44%); the second zone was identified as the dehalogenated N^2-isomer **8b**[13] (21%).

In all cases, the position of glycosylation was deduced from the ^{13}C-NMR-spectra.[12,13] According to the Table (next page) an upfield shift of carbon-3 is observed for the N-2 nucleosides in comparison to the N^1-isomers which show similar chemical shifts for the base moiety as the free nucleobase. The assignment of the ß-D configuration was confirmed by the ^{1}H -NMR spectra using the characteristic shift differences of the H-C(4') and CH_2(5') of the toluoylated compounds.[16]

From the experiments described above, it is apparent that the loss of the 3-bromo substituent occurs only in the case of the N-2 deoxyribofuranosides whereas the bromo substituent is stable in the N-1 compounds **6a** and **6b**. The dehalogenation observed during the anion-glycosylation of **4a** and **4b** in the presence of powdered KOH - containing 15% of water - is not observed under anhydrous condition when NaH is used instead of powdered KOH. In this case the desired 3-bromo isomers **6a** (34%) and **7a** (15%) were formed. Correspondingly, the isomers **6b** (41%) and **7b** (20%) were obtained from **4b**. Due to these observations the dehalogenated compounds **8a** and **8b** have to be formed after glycosylation. This was confirmed in a separate experiment by treatment of the N-2 bromo compounds **7a** or **7b** in a mixture of MeCN / powdered KOH but without the halogenose **5**[14]. Indeed, the bromo nucleosides **7a** and **7b** are converted into the dehalogenated compounds **8a** and **8b**.

According to these findings, various mechanisms can be discussed for the dehalogenation reaction. Nevertheless, the formation of the dehalogenated N-2 isomers **8a** and **8b** being reduced species of the bromo compounds **7a** and **7b** has to be accompanied by the oxidation of another molecule being present in the reaction mixture. A possible route is given below. In this Scheme, the p-quinoid structure of the N-2 isomer undergoes a nucleophilic addition/elimination reaction. In the first step water is added by an initial attack of hydroxyl ions on the most electrophilic center (C-3). This may lead to an intermediate which undergoes a base-catalyzed elimination in a second step thus restoring the p-quinoid system. According to these findings it is apparent that

TABLE. ^{13}C-NMR Chemical Shifts of Pyrazolo[3,4-d]pyrimidines 2'-Deoxyribofuranosides .

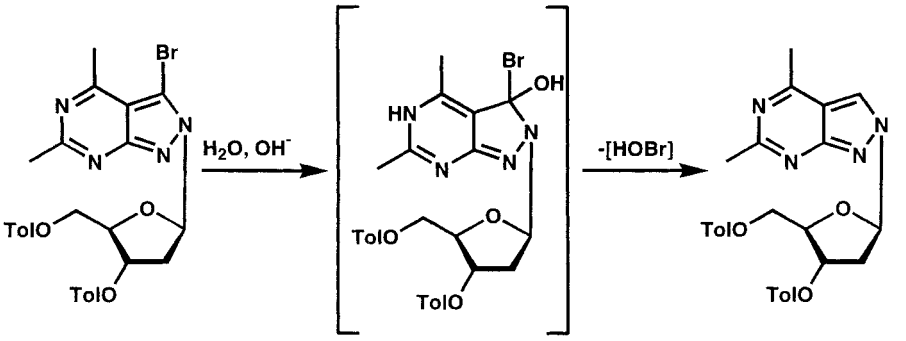
Comp.	C-3	C-3a	C-4	C-6	C-7a	Me	i-Prop
3a	131.1	101.2	163.2	154.5	154.1	53.6	-
3b	131.5	95.8	162.8	162.0	158.9	-	21.7, 68.2
4a	118.1	101.5	163.1	156.1	156.5	54.5	-
4b	118.4	95.5	162.5	162.5	159.5	-	21.8, 68.9
6a	119.0	102.6	163.0	156.0	155.4	54.1	-
6b	120.0	96.3	162.6	162.5	158.7	-	21.6, 64.0
7a	108.9	103.7	[b]	156.1	159.7	54.4	-
7b	108.2	99.2	163.7	161.7	162.4	-	21.1, 69.1
8a	124.8	102.4	[c]	155.5	161.0	54.0	-
8b	12.5	98.3	163.4	161.6	164.2	-	21.1, 68.6

	C-1'	C-2'	C-3'	C-4'	C-5'
6a	84.5	35.0	74.2	81.4	63.4
6b	83.4	34.8	74.9	81.3	64.0
7a	88.2	36.2	74.1	82.3	63.4
7b	87.1	36.0	74.4	81.8	63.8
8a	90.7	37.1	74.5	82.4	64.0
8b	89.3	36.6	74.8	81.8	64.1

[a] Measured in (D_6)DMSO at 23°. [b] Not detectable.

[c] Superimposed by CO.

3-halogenated pyrazolo[3,4-d]pyrimidine N-1 nucleosides can be incorporated into oligonucleotides chemically while the alkaline deprotection conditions employed during the oligonucleotide synthesis cycle do dehalogenate the N-2 nucleoside residues.



EXPERIMENTAL

General. Elemental analyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany). NMR-Spectra were measured on a AC 250 and AMX 500 spectrometer (Bruker, Germany). Chemical shifts are in ppm relative toTMS as internal standard. UV-spectra were recorded on a U 3200 spectrometer (Hitachi, Japan). Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck, Germany). Column chromatography was performed on silica gel 60 (Merck, Germany).

3-Bromo-4-methoxy-1H-pyrazolo[3,4-d]pyrimidine (4a).

To a suspension of **3a**[12] (600 mg, 4 mmol) in 1,2-dichloroethane (50 ml) were added N-bromosuccinimide (1.1 g, 6.2 mmol) and azoisobutyrylnitrile(AIBN, 50 mg). After heating under reflux for 30 min, the solvent was evaporated and the residue applied to a silica gel column. The elution was performed with methylene chloride/methanol (0% → 5% methanol). Crystallization from CH_2Cl_2/MeOH afforded colorless crystals (690 mg, 75%) of **4a:** mp 194-196°C (dec.); TLC (CH_2Cl_2/MeOH, 95:5): R_f 0.4; UV (MeOH): λ_{max} 246, 266 nm (ε = 5200, 3500) ^{1}H-NMR (250 MHz, (D_6) DMSO): δ 4.09 (s, 3H, OCH_3), 8.55 (s, 1H, H-(C6)), 14.26 (s, 1H, NH); Anal. calcd. for $C_6H_5BrN_4O$ (229.0): C 31.47, H 2.20, N 24.46. Found: C 31.26, H 2.28, N 24.49.

6-Amino-3-bromo-4-isopropoxy-1H-pyrazolo[3,4-d]pyrimidine (4b).

Compound **4b** was prepared as described for **4a** but using the following amounts: **3b**[13]

(1.0 g, 5.2 mmol); NBS (1.0 g, 5.6 mmol; 1.5 h). The solution was evaporated to dryness, the residue dissolved in MeOH, decolorized with charcoal and filtered. Upon addition of ice-water a colorless amorphous solid precipitated (1.1 g, 78%). An analytical sample was crystallized from i-PrOH: mp 221°C (dec.); TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.4; UV (MeOH): λ_{max} 248, 276 nm (ε = 6000, 7100); ^{1}H-NMR (250 MHz, (D_6) DMSO): δ 1.32 (d, J = 6.4 Hz, 6H, $(CH_3)_2$), 5.41 (m, 1H, OCH), 6.77 (s, 2H, NH_2), 13.0 (s, 1H, NH); Anal. calcd. for $C_8H_{10}BrN_5O$ (272.1): C 35.31, H 3.70, N 25.74. Found: C 35.58, H 3.81, N 25.54.

Nucleobase Anion-Glycosylation of Compounds 4a and 4b with the Halogenose 5[14] in the Presence of KOH/TDA-1 (Method A):

To a suspension of **4a** (1.0 g, 4.4 mmol) in MeCN (60 ml), KOH (85%, 470 mg, 7.1 mmol) and TDA-1(= tris[2-(2-methoxyethoxy)ethyl]amine; 75 µl) was added. After stirring at r.t. for 10 min **5**[14] (2.1 g, 5.4 mmol) was added and stirring continued for another 20 min. Insoluble material was filtered off and after evaporation the residue was subjected to flash chromatography (FC).

3-Bromo-1-[2-deoxy-3,5-di-O-(p-toluoyl)-ß-D-erythro-pentofuranosyl]-4-methoxy-1H-pyrazolo[3,4-d]pyrimidine (6a).

From the fast migrating main zone (petroleum ether/ethyl acetate 2:1) **6a** was isolated. Crystallization from petroleum ether/ethyl acetate yielded colorless needles (1.13 g, 44%): mp 177-178°C (dec.); TLC (petroleum ether/ethyl acetate, 1:1): R_f 0.6; UV (MeOH): λ_{max} 240, 270 nm (ε = 34300, 7500); ^{1}H-NMR (500 MHz, (D_6) DMSO): δ 2.41, 2.51 (2s, 6H, $2CH_3$), 2.81 (m, 1H, H_α-(C2')), 3.27 (m, 1H, H_β-(C2')), 4.14 (s, 3H, OCH_3), 4.48 (m, 2H, H-(C5')), 4.58 (m, 1H, H-(C4')), 5.81 (m, 1H, H-(C3')), 6.83 ('t', J = 6.2 Hz, 1H, H-(C1')), 7.35, 7.92 (4d, J = 7.8, 7.9 Hz, 8H, $2C_6H_4$), 8.67 (s, 1H, H-(C6)). Anal. calcd. for $C_{27}H_{25}BrN_4O_6$ (581.4): C 55.78, H 4.33, N 9.64. Found: C 55.98, H 4.43, N 9.53.

3-Bromo-2-[2-deoxy-3,5-di-O-(p-toluoyl)-ß-D-erythro-pentofuranosyl]-4-methoxy-1H-pyrazolo[3,4-d]pyrimidine (7a).

From the second zone (petroleum ether/ethyl acetate 1:1) a colorless foam was obtained (205 mg, 8%): TLC (petroleum ether/ethyl acetate, 1:1): R_f 0.4; UV (MeOH): λ_{max} 240 nm (ε = 33900); ^{1}H-NMR (500 MHz, (D_6) DMSO): δ 2.30, 2.35 (2s, 6H, $2CH_3$),

2.82 (m, 1H, H_{α}-(C2')), 3.39 (m, 1H, H_{β}-(C2')), 4.06 (s, 3H, OCH_3), 4.33, 4.48 (2m, 2H, H-(C5')), 4.59 (m, 1H, H-(C4')), 5.90 (m, 1H, H-(C3')), 6.68 (dd, J = 6.3, 6.5 Hz, 1H, H-(C1')), 7.24, 7.80 (4d, J = 7.9, 8.0 Hz, 8H, $2C_6H_4$), 8.56 (s, 1H, H-(C6)). Anal. calcd. for $C_{27}H_{25}BrN_4O_6$ (581.4): C 55.78, H 4.33, N 9.64. Found: C 55.79, H 4.43, N 9.65.

2-[2-Deoxy-3,5-di-O-(p-toluoyl)-ß-D-erythro-pentofuranosyl]-4-methoxy-1H-pyrazolo[3,4-d]pyrimidine (8a).

Evaporation of the slow migrating zone (petroleum ether/ethyl acetate 1:2) yielded a colorless foam of **8a**[12] (110 mg, 5%) upon evaporation. UV (MeOH): λ_{max} 242 nm (ε = 35600) (Lit.[12] 242 nm (ε = 36000)). All other data were identical with the literature.[12]

6-Amino-3-bromo-1-[2-deoxy-3,5-di-O-(p-toluoyl)-ß-D-erythro-pentofuranosyl]-4-isopropoxy-1H-pyrazolo[3,4-d]pyrimidine (6b).

As described for **4a** compound **4b** (1.5 g, 5.5 mmol) was treated in an analogous manner with KOH (85%, 1.46 g, 22 mmol), MeCN (150 ml), TDA-1 (20 µl) and **5**[14] (2.6 g, 6.7 mmol). Column chromatography (CH_2Cl_2/acetone 98:2) gave a colorless foam (1.5 g, 44%): TLC (CH_2Cl_2/acetone 98:2): R_f 0.5; UV (MeOH): λ_{max} 231, 274 nm (ε = 35400, 7700); ^{1}H-NMR (500 MHz, (D_6) DMSO): δ 1.33 (d, J = 6.2 Hz, 6H, $CH(CH_3)_2$), 2.36, 2.38 (2s, 2Ar-CH_3), 2.67 (m, 1H, H_{α}-(C2')), 3.15 (m, 1H, H_{β}-(C2')), 4.50 (m, 3H, H-(C5',C4'), 5.43 (m, 1H, OCH), 5.73 (m, 1H, H-(C3')), 6.55 ('t', J = 5.9 Hz, 1H, H-(C1')), 7.05 (s, 2H, NH_2), 7.28-7.92 (4d, J = 8.1 Hz, 8H, $2C_6H_4$). Anal. calcd. for $C_{29}H_{30}BrN_5O_6$ (624.5): C 55.78, H 4.84, N 11.21. Found: C 56.11, H 4.84, N 10.93.

6-Amino-2-[2-deoxy-3,5-di-O-(p-toluoyl)-ß-D-erythro-pentofuranosyl]-4-isopropoxy-1H-pyrazolo[3,4-d]pyrimidine (8b).

From the second zone compound **8b**[13] (630 mg, 21%) was obtained. UV (MeOH): λ_{max} 224 nm (ε = 42200) (Lit.[13] 224 nm (ε = 42500)). All other data were identical with the literature.[13]

Nucleobase Anion-Glycosylation of Compounds 4a and 4b with the Halogenose 5[14] in the Presence of NaH (Method B):

Glycosylation of **4a**: To a suspension of **4a** (1.0 g, 4.4 mmol) in MeCN (60 ml) was added NaH (97%,163 mg, 6.6 mmol). After stirring at r.t. for 10 min **5**[14] (2.1 g, 5.4 mmol) was added and stirring continued for 30 min. The mixture was filtered and the filtrate

was evaporated. The further work-up was performed as described under Method A. The fast migrating zone furnished compound **6a** (870 mg, 34%). From the second zone compound **7a** was isolated (384 mg, 15%).

The glycosylation of **4b** was performed as described for **4a** using the following amounts: **4b** (1.5 g, 5.5 mmol), NaH (97%, 150 mg, 6.1 mmol), MeCN (100 ml) and **5**[14] (2.6 g, 6.6 mmol). The work-up was the same as described under Method A.

6-Amino-3-bromo-2-[2-deoxy-3,5-di-O-(p-toluoyl)-ß-D-erythro-pentofuranosyl]-4-isopropoxy-1H-pyrazolo[3,4-d]pyrimidine (7b).

Flash-chromatography of the reaction product was performed with CH_2Cl_2/acetone 98:2 yielding two main zones. From the fast migrating zone **6b** was obtained (1.4 g, 41 %). The second zone yielded **7b** as a colorless foam (680 mg, 20%): TLC (CH_2Cl_2/MeOH 95:5): R_f 0.5; UV (MeOH): λ_{max} 268, 303 nm (ε = 11600, 5500); ^{1}H-NMR (500 MHz, (D_6) DMSO): δ 1.33 (d, J = 6.7 Hz, 6H, $(CH_3)_2$), 2.35, 2.38 (2s, 6H, Ar-CH_3), 2.67 (m, 1H, H_α-C(2')), 3.15 (m, 1H, $H_ß$-C(2')), 4.44 (m, 1H, H-C(4')), 4.51 (m, 2H, H-C(5')), 5.43 (m, 1H, OCH), 5.91 (m,1H, H-C(3')), 6.52 ('t', J = 6.2 Hz, 1H, H-C(1')), 6.64 (s, 2H, NH_2), 7.28-7.92 (4d, J = 8.1 Hz, 8H, $2C_6H_4$). Anal. calcd. for $C_{29}H_{30}BrN_5O_6$ (624.5): C 55.78, H 4.84, N 11.21. Found: C 55.89, H 4.80, N 11.24.

Debromination of Compounds 7a and 7b in MeCN/Powdered KOH:

To a solution of compound **7a** (200 mg, 0.34 mmol) in MeCN (30 ml) powdered KOH (85%, 240 mg, 3.6 mmol) was added. The suspension was stirred for 30 min at r.t., filtered and the filtrate was evaporated. The residue was subjected to FC (petroleum ether/ethyl acetate 1:1) yielding compound **8a** as a colorless foam (60 mg, 35%). The debromination of compound **7b** was performed analogously furnishing compound **8b**.

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