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A practical approach for the chemical synthesis of 2′-deoxyguanosine-C8 adducts with mutagenic/carcinogenic amino- or nitro-arenes

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Abstract—Synthetic methods for the preparation of 2′-deoxyguanosine-C8 (dG-C8) adducts with several mutagenic and carcinogenic amino- or nitro-arenes were developed using the palladium-mediated cross-coupling reaction of protected 8-amino-dG with bromoarenes in around 80% yields, followed by conventional deprotection procedures. This approach can be applied to preparation of a variety of authentic dG-C8 adducts with amino or nitro-arenes.

Aromatic amino or nitro compounds are widely distributed in our environment, being present in cooked foods, cigarette smoke, airborne particles, surface soil, river water, industrial materials and diesel exhaust particulates.[1] Some examples are mutagenic and/or carcinogenic and are suspected of involvement in human cancer development.[2] Based on a number of studies, it is now generally recognized that one of the first crucial steps for carcinogenesis is the covalent modification of DNA with active metabolites derived from mutagens and carcinogens.[2,3] Both aromatic amino and nitro compounds give rise to *N*-hydroxylamine intermediates as activated metabolites, either through N-oxidation of the primary aryl amines by cytochrome P450s or sequential reduction of nitroarenes by reductase.[2b,3b,c] *N*-Hydroxylamines are further *O*-esterified by the action of *O*-acetyltransferase or sulfotransferase and the resultant activated esters readily undergo heterolytic N–O cleavage to form highly reactive nitrenium ions, which attack DNA. They covalently bind predominantly at the C8 position of 2′-deoxyguanosine (dG) through the exocyclic N atom and to a lesser extent at the N^2 of dG through the aromatic C atom, the compounds tending to be cationic through resonance stabilization from the initial nitrenium ion intermediate.[3]

With the aid of authentic dG-C8 DNA adducts prepared with *N*-acyloxyarylamine, chemical structural analyses and quantification have been performed for DNA adducts formed in vivo with mutagenic/carcinogenic amino- or nitro-arenes.[2,3] However, their biological effects on capability to induce mutations and DNA repair have not yet been fully clarified due to the difficulty of synthesis of site specific adducted DNA by the *N*-acyloxyarylamine methods, which demand tremendous repeated HPLC separation–fractionation.[4] Synthesis of DNA-oligomers containing adduct(s) at a definite site at good yield would provide a valuable tool for elucidating the structural and functional properties of damaged DNA. Moreover, clarification of the nature of many unidentified DNA adducts in human tissue samples might be facilitated, if authentic dG-C8 adducts were available. Thus, a reliable system for high yield preparation of modified nucleotides, especially dG-C8 adducts with amino- or nitro-arenes is required.

The Buchwald–Hartwig amination reaction has been used for the synthesis of a wide variety of nucleoside derivatives.[5] Some dG-C8 adducts have been synthetically obtained from protected 8-bromo-dG with aminoarenes.[6] In order to apply palladium-catalyzed arylamination reactions to the synthesis of dG-C8 adducts, appropriate protection of the amino group at the 2 position of dG is needed. Wang and Rizzo used STABASE and BisBOC protective groups.[6a] More recently, Gillet and Scharer reported that a 4,4′-

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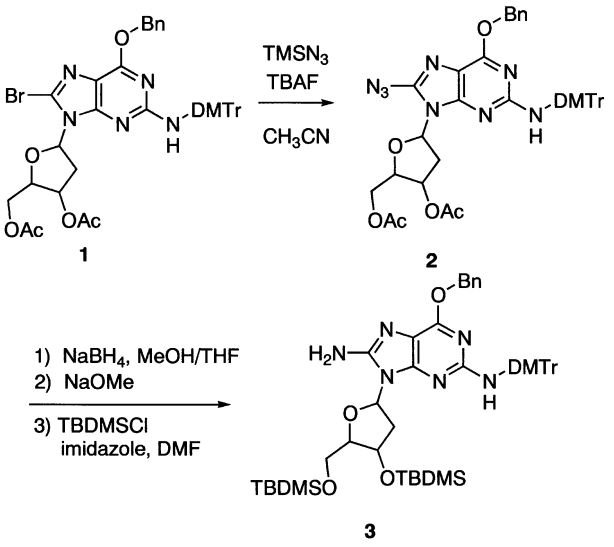
dimethoxytrityl (DMTr) group for N^2 protection of dG is compatible with the coupling of 8-bromo-dG and arylamine.[6b] In every cases, the BINAP ligand was used for the successful coupling reaction, however, the reaction yield varies and is somewhat lower when 1-aminopyrene is used as a substrate.

We report here more precise, definite and practical methods to obtain dG-C8 adducts with amino- or nitro-arenes. Our synthetic approach features the palladium mediated coupling method using N^2,O-protected 8-amino-dG and bromoarene. This is an inverse procedure which has been reported for N^2,O-protected 8-bromo-dG and aminoarene as substrates.[5] Several coupling reactions of aminonucleosides with bromoarenes have already been reported but there are no applications to 8-amino-dG.[5] In the present study, we focused on the synthesis of DNA adducts with mutagenic/carcinogenic compounds, such as 4-aminobiphenyl,[2c] β-napthylamine,[2c] 2-aminofluorene,[2c] 1-nitropyrene[2c] and 3-nitrobenzanthrone.[7]

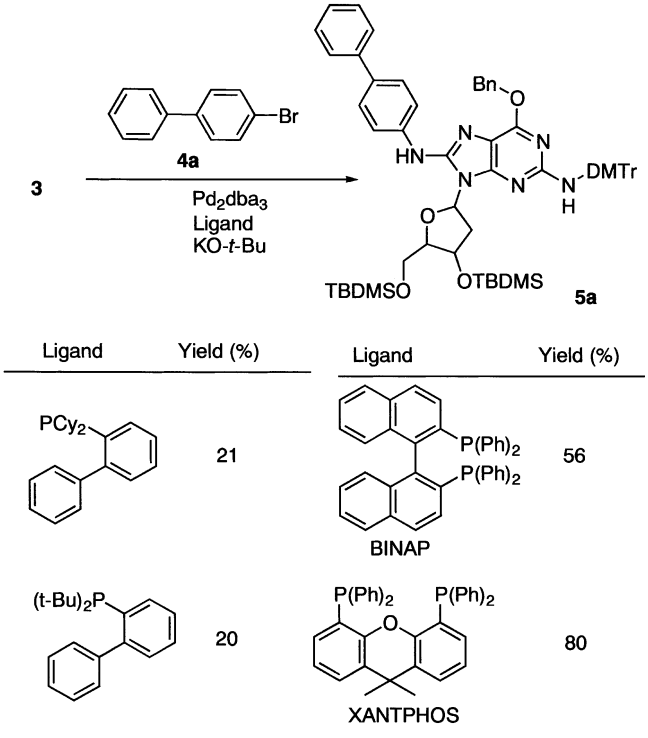
Synthesis of 8-amino-dG is the critical step for success with this approach. The reported method to yield 8-amino-dG is treatment of 8-bromo-dG with hydrazine hydrate, followed by reduction of the resulting 8-hydrazido-dG.[8] In our hands, however, this method generated large amounts of debromination product, i.e. dG, and was not suitable for obtaining appreciable amounts of 8-amino-dG. Moreover, aqueous alkaline reaction conditions would make it impossible to use conventional protective groups for OH and NH_2 functional groups. We therefore established an alternative method to produce 8-amino-dG using the $TMSN_3$ and TBAF system.[9] This method is attractive because the use of $TMSN_3$ and TBAF might not affect the acyl protective group generally used for the protection of dG. N^2-DMTr-3′,5′-bis-O-acetyl-O^6-benzyl-8-bromo-dG **1**, prepared routinely, was treated with 10 equiv. of $TMSN_3$ and TBAF in acetonitrile. Azidation proceeded smoothly and was completed within 8 h at 65°C (Scheme 1). The reaction was clear and no by-products were observed on TLC analyses. Azide **2** was not stable under light, so it was immediately reduced and subsequently deacetylated to the corresponding amine by $NaBH_4$ followed by NaOMe treatment in the methanol/THF system.[10] After silyl protection of 3′,5′-OH groups of the deoxyribose moiety, compound **3** was subjected to a palladium coupling reaction with 4-bromobiphenyl **4a**.

The fully protected amine **3** was treated with bromobiphenyl **4a** by palladium-mediated cross-coupling using BINAP, 10 mol% of Pd_2dba_3 and KO-t-Bu as a base at 100°C. Under these reaction conditions, a cross-coupling product **5a** was obtained at around a 10% yield. More load of palladium (20 mol%) increased the yield up to 52%. Biphenyl phosphine ligands, recently developed by the Buchwald group,[11] have been used for arylamination of nucleosides. Several types of biphenyl ligands containing a dicyclohexylphosphino group, a di-t-butyl phosphino group and/or an N,N-dimethylamino group, were employed but did not improve the yield of the coupled product (Scheme 2). 1,1′-Bis-(diphenylphosphino)ferrocene and tri-t-butyl phosphine was not effective. In contrast, XANTPHOS was shown to be an excellent ligand with our synthetic strategy. Using XANTPHOS, 1.2 equiv. to Pd(0), coupling compound **5a** was obtained at an 80% yield (Scheme 2).[12]



Scheme 1.

A DMTr group at N^2 protective group was tolerable under our reaction conditions. However, coupling reactions using a dimethylformamide amidine or a triphenylphosphine imide as a protective group of N^2 of dG failed, probably due to the lower compatibility under alkaline conditions. Moreover, N^2-isobutylyl



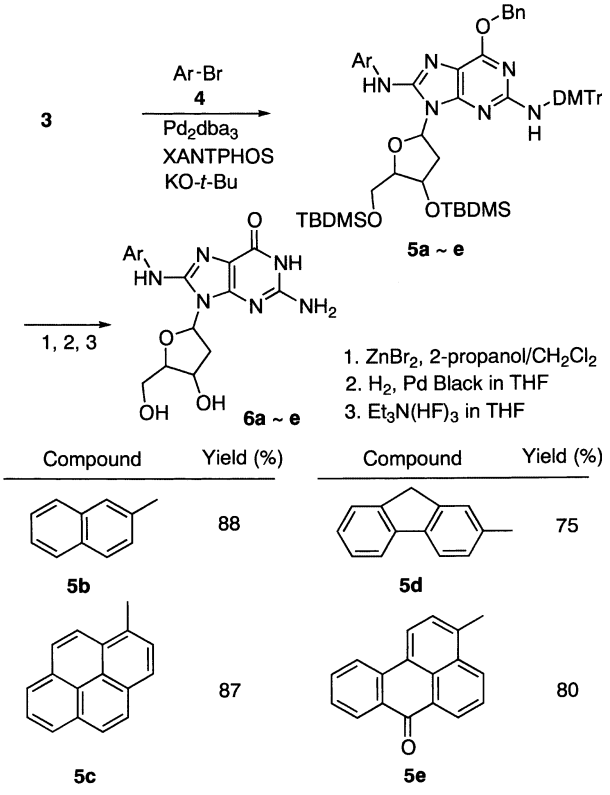
Scheme 2.

protection was not suitable and coupling reactions did not occur, although several studies have demonstrated that amide functional groups are compatible and can even be used as substrates.[6c]

Similarly, four other bromoarenes **4b–e** were found to effectively couple with **3** at yields of 75–88% (Scheme 3). 1-Bromopyrene **4c** and 3-bromobenzanthrone **4e** also coupled with **3** using BINAP as the phosphine ligand in around 80%.[12] The coupling yields obtained in this study were generally higher than those using protected 8-bromo-dG and aminoarenes as substrates for the palladium mediated arylamination reaction previously reported.[5b]

Further deprotection procedures to obtain authentic dG-C8 adducts were examined (Scheme 3). Detritylation of **5a–d** was performed with trifluoroacetic acid in CH_2Cl_2 at 0°C. Although acidic conditions may cause depurination of amino nucleosides, careful treatment of trifluoroacetic acid minimized the depurinating by-products and the desired products were obtained in moderate yields (70%).

A more selective detritylation procedure for **5a–e** was by treatment of $ZnBr_2$ in 2-propanol/CH_2Cl_2 as the solvent. This deprotection procedure caused little depurination products and the desired compound was obtained in around a 90% yield in each case.[13] Quantitative removal of the benzyl group was performed with hydrogenation using Pd black/H_2 at room temperature. The TBDMS group was removed by treatment with triethylamine trihydrogenfluoride ($Et_3N(HF)_3$).[14]



Scheme 3.

Desired adducts **6a–e** were obtained almost quantitatively. Conventional TBAF treatment was not useful, resulting in the formation of many unidentified by-products.

We describe here methods to obtain dG-C8 adducts with amino- or nitro-arenes. Some types of bromoarenes can be easily obtained by one-step bromination of parent polyaromatic hydrocarbons. Therefore, the approach with palladium coupling methods using the 8-aminonucleoside route is very useful to obtain dG-C8 adducts and may feasibly be employed to prepare many types of dG-C8 adducts. Generation of authentic samples would be very useful to identify unknown DNA adducts detected in human tissues. Moreover, this approach can be applied to synthesis of site specific adducted oligo-DNA.

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10. To compound **1** (2 mmol) dissolved in 20 ml of acetonitrile, trimethylsilyl azide (10 mmol) was added via a syringe followed by addition of 10 ml of 1 M solution of TBAF in THF. The reaction was heated at 65°C for 8 h and the mixture was then allowed to return to room temperature and concentrated in vacuo to give a yellow oil which was chromatographed (hexane:ethyl acetate, 3:1) to afford **2** as a colorless oil; FAB-HRMS m/z: 785.3096 (calcd for $C_{42}H_{41}N_8O_8$: 785.3057), IR ν (cm^{-1}): 2148 (N_3). This oil was immediately dissolved in methanol and to this solution was added $NaBH_4$ (50 mg) at 0°C. After the initial spot on TLC disappeared, sodium methoxide (300 μl) was added. The reaction mixture was quenched by the addition of water and extracted with ethyl acetate. The organic extract was combined, dried over Na_2SO_4, and filtered. The solvent was finally removed in vacuo to yield almost pure desired amine (45% yield from **1**). ^1H NMR ($CDCl_3$, 600 MHz) δ: 7.31–7.16 (m, 14H), 6.74 (d, $J=8.8$, 4H), 6.05 (brs, 2H), 5.10 (brs, 2H), 4.75 (brs, 1H), 4.54 (brs, 1H), 3.92–3.67 (m, 9H), 2.77 (brs, 1H), 2.01 (brs, 1H): FAB-HRMS: m/z: 675.2977 ([M+H]$^+$) (calcd for $C_{38}H_{39}N_6O_6$: 675.2931).
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12. Amine **3** (0.1 mmol), arylbromide **4** (0.12 mmol), potasium *tert*-butoxide (0.15 mmol), Pd_2dba_3 (0.01 mmol), and a phosphine ligand (0.024 mmol) were placed in a 5 ml flask and purged with argon. Toluene (1 ml) was added and the reaction vessel was heated to 100°C for 2 h. The mixture was cooled to 25°C, diluted with chloroform and filtered through Celite. The filtrate was concentrated and purified by column chromatography on silica gel. **5a**: ^1H NMR ($CDCl_3$, 600 MHz) δ: 7.61 (d, $J=8.2$, 1H), 7.55 (d, $J=7.1$, 1H), 7.49 (d, $J=8.2$, 1H), 7.42 (s, 1H), 7.39 (t, $J=8.2$, 1H), 7.34–7.15 (m, 18H), 6.74 (d, $J=8.8$, 4H), 6.19 (brs, 1H), 6.03 (s, 2H), 4.45 (brs, 1H), 4.00–3.77 (m, 3H), 3.76 (s, 6H), 2.70 (brs, 1H), 2.10 (brs, 1H), 0.92 (s, 3H), 0.90 (s, 6H), 0.85 (s, 6H), 0.81 (s, 3H), 0.11 (s, 2H), 0.08 (s, 2H), 0.07 (s, 2H), 0.04 (s, 2H), 0.03 (s, 2H), 0.05 (s, 1H), 0.07 (s, 1H): FAB-HRMS: m/z 1055.5236 ([M+H]$^+$) (calcd for $C_{62}H_{75}N_6O_6Si_2$: 1055.5287). **5b**: ^1H NMR ($CDCl_3$, 600 MHz) δ: 8.12 (s, 1H), 7.77–7.69 (m, 4H), 7.52 (dd, $J=8.7$, 2.1, 2H), 7.40–7.17 (m, 14H), 6.74 (d, $J=6.6$, 4H), 6.19 (brs, 1H), 6.04 (s, 2H), 5.09 (brs, 1H), 4.49 (brs, 1H), 4.00–3.79 (m, 3H), 3.76 (s, 6H), 2.60 (brs, 1H), 2.10 (brs, 1H), 0.89 (s, 9H), 0.87 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H): FAB-HRMS: m/z 1029.5177 ([M+H]$^+$) (calcd for $C_{60}H_{73}N_6O_6Si_2$: 1029.5130). **5c**: ^1H NMR ($CDCl_3$, 600 MHz) δ: 8.57 (brs, 1H), 8.41 (brs, 1H), 8.16–8.10 (m, 4H), 8.05–7.94 (m, 4H), 7.34 (d, $J=7.14$, 2H), 7.33–7.17 (m, 12H), 6.76 (d, $J=8.2$, 4H), 6.24 (brs, 1H), 6.04 (s, 2H), 5.02 (brs, 1H), 4.51 (brs, 1H), 4.12 (m, 1H), 3.91–3.75 (m, 8H), 2.22 (brs, 1H), 1.64 (brs, 1H), 0.92 (s, 9H), 0.58 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H), –0.22 (s, 3H), –0.34 (s, 3H): FAB-HRMS: m/z 1103.5319 ([M+H]$^+$) (calcd for $C_{66}H_{75}N_6O_6Si_2$: 1103.5287). **5d**: ^1H NMR ($CDCl_3$, 600 MHz) δ: 7.88 (s, 1H), 7.70–7.63 (m, 3H), 7.48 (d, $J=7.7$, 1H), 7.43 (d, $J=8.3$, 1H), 7.35–7.15 (m, 17H), 6.75 (d, $J=9.3$, 4H), 6.12 (brs, 1H), 6.04 (s, 2H), 5.02 (brs, 1H), 4.48 (brs, 1H), 4.00–3.80 (m, 5H), 3.85 (s, 6H), 2.64 (brs, 1H), 2.12 (brs, 1H), 0.90 (s, 9H), 0.86 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H): FAB-HRMS: m/z 1067.5310 ([M+H]$^+$) (calcd for $C_{63}H_{75}N_6O_6Si_2$: 1067.5286). **5e**: ^1H NMR ($CDCl_3$, 600 MHz) δ: 8.78 (d, $J=7.1$, 1H), 8.47 (d, $J=7.1$, 1H), 8.43 (brs, 2H), 8.29 (d, $J=8.3$, 1H), 8.27 (d, $J=8.3$, 1H), 7.75 (t, $J=7.7$, 1H), 7.70 (t, $J=7.1$, 1H), 7.48 (t, $J=7.7$, 1H), 7.34 (d, $J=7.6$, 2H), 7.33–7.17 (m, 12H), 6.76 (d, $J=8.2$, 4H), 6.10 (brs, 1H), 6.08 (s, 1H), 5.02 (brs, 2H), 4.44 (brs, 1H), 4.14 (brs, 1H), 3.91–3.75 (m, 8H), 2.22 (brs, 1H), 1.64 (brs, 1H), 0.91 (s, 9H), 0.61 (s, 9H), 0.099 (s, 6H), –0.19 (s, 3H), –0.31 (s, 3H): FAB-HRMS: m/z 1103.5319 ([M+H]$^+$) (calcd for $C_{67}H_{75}N_6O_7Si_2$: 1103.5287).
13. Fully protected dG adduct **5a**–**e** (0.1 mmol) was dissolved in dichloromethane (2 ml). To this solution was added 1 ml of 1 M zinc bromide solution in 2-propanol/dichloromethane. The reaction was quenched by the addition of aqueous $NaHCO_3$, and materials were extracted with dichloromethane. The organic layer was combined, dried and evaporated, and the residue was chromatographed on silica gel using hexane–ethylacetate as an eluent.
14. Detritylated compounds **5a**–**e** (0.05 mmol) in THF were hydrogenized under a balloon with palladium black (20 mg). After 30 min, Pd black was filtered off with Celite, and the filtrate was evaporated. The residue was redissolved in THF and triethylamine trihydrogenfluoride (100μl) was added to this solution with stirring overnight at room temperature. The next day, triethylamine (88 μl) was added and the solvent was evaporated. The residue was dissolved in CH_3CN/water and subjected to HPLC with an ODS column. Elution was performed with a linear gradient of 15% to 80% acetonitrile containing 0.25% triethylamine-acetate buffer at pH 7.0. A main peak was collected and freeze-dried. **6a**: ^1H NMR (DMSO-d_6, 600 MHz) δ: 10.56 (brs, 1H), 8.75 (s, 1H), 7.82 (d, $J=8.8$, 2H), 7.63 (d, $J=7.7$, 2H), 7.58 (d, $J=8.8$, 2H), 7.43 (d, $J=7.1$, 1H), 7.42 (d, $J=7.7$, 1H), 7.29 (t, $J=7.1$, 1H), 6.39 (s, 2H), 6.37 (dd, $J=9.9$, 6.0, 1H), 5.97 (s, 1H), 5.33 (s, 1H), 4.42 (d, $J=5.5$, 1H), 3.93 (s, 1H), 3.77 (s, 2H), 2.41 (dd, $J=17.3$, 7.1, 1H), 2.02 (dd, $J=13.2$, 6.0): FAB-HRMS: m/z: 435.1781 ([M+H]$^+$) (calcd for $C_{22}H_{23}N_6O_4$: 435.1781). **6b**: ^1H NMR (DMSO-d_6, 600 MHz) δ: 10.57 (brs, 1H), 8.83 (s, 1H), 8.34 (s, 1H), 7.81–7.72 (m, 4H), 7.42 (t, $J=7.7$ 1H), 7.31 (t, $J=7.7$, 1H), 6.37–6.35 (m, 3H), 6.00 (t, $J=4.4$, 1H), 5.33 (d, $J=3.3$, 1H), 4.44 (s, 1H), 3.94 (d, $J=1.6$, 1H), 3.79 (s, 2H), 2.79 (ddd, $J=13.2$, 9.9, 6.0, 1H), 2.35 (dd, $J=12.6$, 6.1, 1H): FAB-HRMS: m/z: 409.1624 ([M+H]$^+$) (calcd for $C_{20}H_{21}N_6O_4$: 409.1624). **6c**: ^1H NMR (DMSO-d_6, 600

MHz) δ: 10.52 (s, 1H), 8.99 (s, 1H), 8.23–8.21 (m, 4H) 8.14–8.02 (m, 5H), 6.36 (dd, $J=9.3$ 6.3, 1H), 6.33 (s, 2H), 5.46 (s, 1H), 5.30 (d, $J=3.8$, 1H), 4.42 (s, 1H), 3.95 (d, $J=2.7$, 1H), 3.79–3.76 (m, 1H), 3.68–3.65 (m, 1H), 2.87 (ddd, $J=13.2$, 9.3, 6.0, 1H), 2.02 (ddd, $J=13.2$, 6.0, 1.9, 1H): FAB-HRMS: m/z 483.1802 ([M+H]$^+$) (calcd for $C_{26}H_{23}N_6O_4$: 483.1781). **6d**: ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 10.55 (s, 1H), 8.74 (s, 1H), 8.09 (s, 1H), 7.77 (d, $J=7.7$, 1H), 7.65 (d, $J=8.2$, 1H), 7.66 (dd, $J=8.2$, 1.1, 1H), 7.52 (d, $J=7.7$, 1H), 7.33 (t, $J=7.7$, 1H), 7.22 (dt, $J=7.7$, 1.1, 1H), 6.36 (s, 2H), 6.34 (t, $J=6.6$, 1H), 5.97 (t, $J=3.3$, 1H), 5.33 (d, $J=3.3$, 1H), 4.43 (s, 1H), 3.93 (d, $J=2.1$, 1H), 3.89 (s, 2H), 3.78 (s, 2H), 2.57–2.53 (m, 1H), 2.02 (dd, $J=12.3$ 5.8, 1H): FAB-HRMS: m/z 447.1820 ([M+H]$^+$) (calcd for $C_{23}H_{23}N_6O_4$: 447.1781). **6e**: ^{1}H NMR (DMSO-d_6, 600 MHz) δ:10.61 (s, 1H), 9.11 (s, 1H), 8.69 (d, $J=7.6$, 2H), 8.66 (d, $J=8.3$, 1H), 8.53 (d, $J=8.3$, 1H), 8.32 (d, $J=8.8$, 1H), 7.90 (t, $J=7.7$, 1H), 7.83 (t, $J=7.7$, 1H), 7.73 (d, $J=7.7$, 1H), 7.57 (t, $J=7.7$, 1H), 6.37 (s, 2H), 6.32 (t, $J=6.6$, 1H), 5.33 (s, 1H), 5.25 (d, $J=3.8$, 1H), 4.38 (brs, 1H), 3.89 (brs, 1H), 3.70 (m, 1H), 3.60 (m, 1H), 2.8 (m, 1H), 2.13 (m, 1H): FAB-MS: m/z 511.3 ([M+H]$^+$).