biphasic kinetic results were observed when analogous experiments were carried out in vesicles of $(n\text{-}C_{20}H_{41})_2N^+Me_2Br^-$, instead of DODAC.

In the preceeding experiments, the molar ratio of Ellman's reagent to surfactant was 1:10. It is important to note that the original observation of a single, fast cleavage of Ellman's reagent (after subsequent addition of this reagent to the performed vesicles) *can* be recreated if the Ellman's/surfactant ratio is adjusted to 1:100. In these experiments, the final concentrations are $[S_2O_4^{2-}] = 5 \times 10^{-4}$ M, [Ellman's] = 2.5×10^{-5} M, and [surfactant] = 2.5×10^{-3} M. With both DODAC and the di-C_{20} vesicles, only a single rapid reaction is observed upon addition of dithionite, even after aging the Ellman's/vesicle solution for 15–30 min. In contrast, when the Ellman's reagent is *cosonicated* with the surfactant before the dithionite addition, both fast and slow ($k < 1 \times 10^{-3}$ s^{-1}) kinetic phases are observed, with the slow phase accounting for ~70% of the total reaction. These phenomena are in general agreement with those reported in reference 2.

We are uncertain why we cannot precisely reproduce the reported phenomena[2] at the original concentrations. In the current 1:10 experiments, we believe that not all of the added Ellman's reagent can be bound at the exovesicular surface. Accordingly, the initial, fast, exovesicular reduction of bound Ellman's reagent is followed by a slower product–desorbtion rate-limiting cleavage of the excess (aqueous) Ellman's reagent as it diffuses to the vesicular surface. At the higher, 1:100 Ellman/surfactant dilution, added Ellman's reagent can be adsorbed at the exovesicular surface, and, upon dithionite addition, it cleaves in a single, fast, uniphasic process. When the Ellman's reagent is cosonicated with the surfactant, separate fast and slow reactions are seen for exo- and endovesicularly bound (or intercalated) Ellman's reagent.

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Registry No. DODAC, 107-64-2; H-Cys-OH, 52-90-4; $Na_2S_2O_4$, 7775-14-6; Ellman's reagent, 69-78-3.

$Cr(ClO_4)_2$: An Effective Reagent for the Preparation of Mitomycin C Nucleophilic Substituted Compounds

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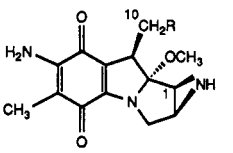
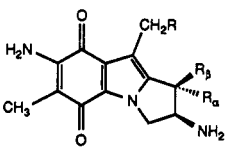
Advances in determining the mode of action of mitomycin C[1,2] have been achieved by the preparation and characterization of select mitomycin C-derived products.[1-10] Recently, we reported that addition of $Cr(ClO_4)_2$ to aqueous and methanolic solutions of **1a** led to the efficient activation of the C(1) and C(10) positions in the anticancer agent.[2i,j] In this paper, we describe the application of this novel reductive activation technique for the generation of mitomycin nucleophilic substituted products. The facility of these reactions provides promise that this method will find use in future mitomycin-based studies.



1a R = $OC(O)NH_2$

b R = OH



2a R_α = H, R_β = H, R = NHPh

b R_α = NHPh, R_β = H, R = NHPh

c R_α = OCH_3, R_β = H, R = OCH_3

d R_α = H, R_β = OCH_3, R = OCH_3

e R_α = H, R_β = H, R = OCH_3

f R_α = OH, R_β = H, R = $OC(O)NH_2$

g R_α = H, R_β = OH, R = $OC(O)NH_2$

h R_α = H, R_β = H, R = $OC(O)NH_2$

i R_α = OH, R_β = H, R = OH

j R_α = H, R_β = OH, R = OH

k R_α = H, R_β = H, R = OH

Results and Discussion

a. Use of Aniline. Addition of 2 equiv of $Cr(ClO_4)_2$ to a buffered methanolic solution (Tris·HCl, "pH" 7.00) containing **1a** and aniline (5 equiv) led to the production of **2a** and **2b**, along with the known adducts **2c–2e**,[2i,j,5e,11a] and several unidentified compounds. HPLC analysis of the reaction mixture indicated that the aniline-bound adducts **2a** and **2b** accounted for approximately 66% of the reaction mixture. Repetition of this experiment on a semipreparative scale permitted the isolation and structural characterization of the two new compounds as 10-decarbamoyl-10-anilino-2,7-diaminomitosene (**2a**) and *trans*-10-decarbamoyl-1,10-dianilino-2,7-diaminomitosene (**2b**). Key ^{1}H NMR resonances detected for **2a** included the diagnostic doublets of doublets located at δ 2.37 and 2.85 for the C(1) methylene protons, the doublet (J = 5.7 Hz) at δ 4.27 for the C(10) methylene hydrogens, the triplet (J = 5.7 Hz) at δ 5.86 for the aniline N–H proton, and the

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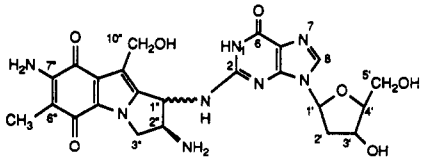
five proton multiplet at δ 6.47–7.06 for the aromatic ring. Correspondingly, in the C(1), C(10)-disubstituted adduct **2b** the C(1) methine proton was observed as a doublet of doublets at δ 4.46 (J = 2.3, 7.4 Hz). The smaller coupling (J = 2.3 Hz) has been assigned to the C(1)H–C(2)H proton–proton interaction. This value is consistent with the proposed trans-disubstitution pattern.[3b,c,5a,b,e] Other notable resonances included the two aniline N–H proton signals at δ 5.42 (t, J = 5.6 Hz) and 5.94 (d, J = 7.4 Hz) and the aromatic peaks located at δ 6.45–7.15, which integrated for 10 hydrogens. Attempts to confirm the identities of **2a** and **2b** by mass spectrometry (+/− CI) were unsuccessful. Use of water (Tris·HCl, pH 7.00) in place of methanol gave comparable results in which compounds **2a** and **2b** were the major products.

b. Use of 2′-Deoxyguanosine. Addition of $Cr(ClO_4)_2$ (2 equiv) to an aqueous buffered solution (Tris·HCl, pH 7.40) containing mitomycin C and 2′-deoxyguanosine (5 equiv) gave a complex product mixture. HPLC analysis indicated the presence of the six known mitosene solvolytic products **2f–2k**[2i,j,4,5e,11,12] and five additional peaks (retention times: 18.1, 18.4, 19.4, 19.6, 22.0 min) that strongly absorbed at both 280 and 313 nm in the ultraviolet spectrum. Previous studies have demonstrated that this absorption pattern is characteristic of mitomycin C–deoxyguanosine adducts.[6] Attempts to satisfactorily separate the component mixture after performing the reaction on a semipreparative scale, however, were unsuccessful.

The complexity of the mitomycin C–deoxyguanosine reaction prompted our use of 10-decarbamoylmitomycin C (**1b**) in place of mitomycin C (**1a**). We suspected that the removal of the C(10) carbamate group would reduce the number of mitomycin C-solvolytic and mitomycin C-deoxyguanosine products generated by eliminating the C(10) position as a likely reaction site. In agreement with this hypothesis, only five prominent peaks were detected in the HPLC chromatogram after treatment of an aqueous buffered solution (Tris·HCl, pH 8.15) containing **1b** and 2′-deoxyguanosine (5 equiv) with $Cr(ClO_4)_2$ (1 equiv). Three of these corresponded to the solvolytic products **2i–2k**. The remaining two peaks were located at 18.2 and 19.0 min and represented approximately 22% of the reaction mixture. Both the 18.2 and 19.0 min signals strongly absorbed at 280 and 313 nm in the ultraviolet spectrum and were present in a 2:1 relative ratio.

Repetition of this reaction on a semipreparative scale permitted our assignment of the HPLC 19.0-min adduct as N^2-(10″-decarbamoyl-2″β,7″-diaminomitosen-1″β-yl)-2′-deoxyguanosine (**3a**).[6c] Several important spectral



3a 1″β
b 1″α

properties were observed for this compound. First, the UV–vis spectrum contained prominent absorptions at 280 and 313 nm consistent with the presence of both the deoxyguanosine and the mitosene chromophores, respectively. Second, key resonances for the mitosene and the deoxyguanosine units were detected in the ^1H NMR spectrum. In particular, we noted the sharp singlet at δ 7.94, the apparent triplet at δ 6.17, and the two sets of multiplets centered at δ 2.23 and 2.54. These values closely matched the resonances observed for 2′-deoxyguanosine and have been assigned to the C(8) ring hydrogen in the guanine ring and the C(1′) and the two C(2′) methylene hydrogens of the sugar unit, respectively. In addition to these resonances, several characteristic signals for the substituted mitosene were clearly evident. Among these were the C(10″) methylene hydrogens at δ 4.50 and 4.54 as an AB quartet, the C(6″) methyl hydrogens at δ 1.74, and the two doublets of doublets at δ 3.91 (J = 4.7, 12.7 Hz) and 4.33 (J = 6.5, 12.7 Hz), which have been attributed to the C(3″)H_β and C(3″)H_α protons, respectively. Further analysis of the ^1H NMR spectrum indicated that the C(1″) methine hydrogen of the mitosene resonated at δ 5.31 as a doublet (J = 6.2 Hz). This coupling constant is consistent with the proposed cis orientation,[3b,c,5a,b,e] and the observed chemical shift value closely matched that reported by Tomasz and co-workers for the corresponding C(10″)-carbamoyl derivative.[6b] Further confirmation of these assignments was provided by the COSY spectrum (Figure 1) for **3a**.[13] Third, analysis of the ^{13}C NMR spectrum for **3a** permitted the assignment of most of the observed resonances. The upfield signals at δ 61.09, 69.91, 81.68, and 86.47 matched those detected for 2′-deoxyguanosine and have been attributed to the C(5′), C(3′), C(4′), and C(1′) carbons in **3a**, respectively. The remaining five upfield signals at δ 8.94, 50.59, 52.71, 53.87, and 56.08 have been assigned to the C(6″) methyl, C(3″), C(2″), C(10″), and C(1″) carbons in the mitosene unit, respectively. Significantly, the upfield values for the C(1″), C(2″), and C(3″) resonances are consistent with previous trends for cis-disubstituted mitosene adducts.[2j,5e]

Structural identification of the major 2′-deoxyguanosine–mitosene 18.2-min adduct was more difficult. Two experimental factors complicated this analysis. First, substantial amounts of the product were lost during the purification process due to the selective precipitation of the 18.2-min adduct at the head of the Sephadex column. Second, the ^1H NMR signals observed for the 18.2-min adduct were noticeably broader than those detected for **3a**. Elevation of the probe temperature from 30 to 45 °C led to a slight increase in the resolution of the spectrum. Despite these inherent problems, key resonances were clearly evident in the ^1H NMR spectrum which permitted us to tentatively assign this compound as N^2-(10″-decarbamoyl-2″β,7″-diaminomitosen-1″α-yl)-2′-deoxyguanosine (**3b**).[6c] In particular, we have attributed the signals at δ 7.83, 6.16, 2.55, and 2.18 to the C(8) guanine proton, the C(1′) methine hydrogen, and the two C(2′) methylene protons of the 2′-deoxyguanosine unit, respectively, and the peaks at δ 4.51, 4.41, 3.82, and 1.73 to the C(10″) methylene hydrogens, the C(3″)H_α and H_β protons, and the C(6″) methyl hydrogens of the mitosene ring system, respectively. Finally, the broad singlet located at δ 4.92 has been tentatively assigned to the C(1″)H_β proton of the mitosene unit. This value is comparable to the resonance (δ ~4.95) previously attributed to the C(1) methine proton in the corresponding C(10″)-carbamoyl derivative.[6b] Attempts to confirm the identities of **3a** and

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(13) No coupling interactions were observed between the C(1″) methine hydrogen of the mitosene unit and the N(2) amino proton of the 2′-deoxyguanosine residue in **3a**. This proton–proton interaction was previously observed for the corresponding C(10″)-carbamoyl derivative.[6b] The absence of this coupling suggests that either the amino proton in **3a** is rapidly undergoing exchange under the conditions of the NMR experiment or **3a** exists predominantly in the tautomeric imino form.

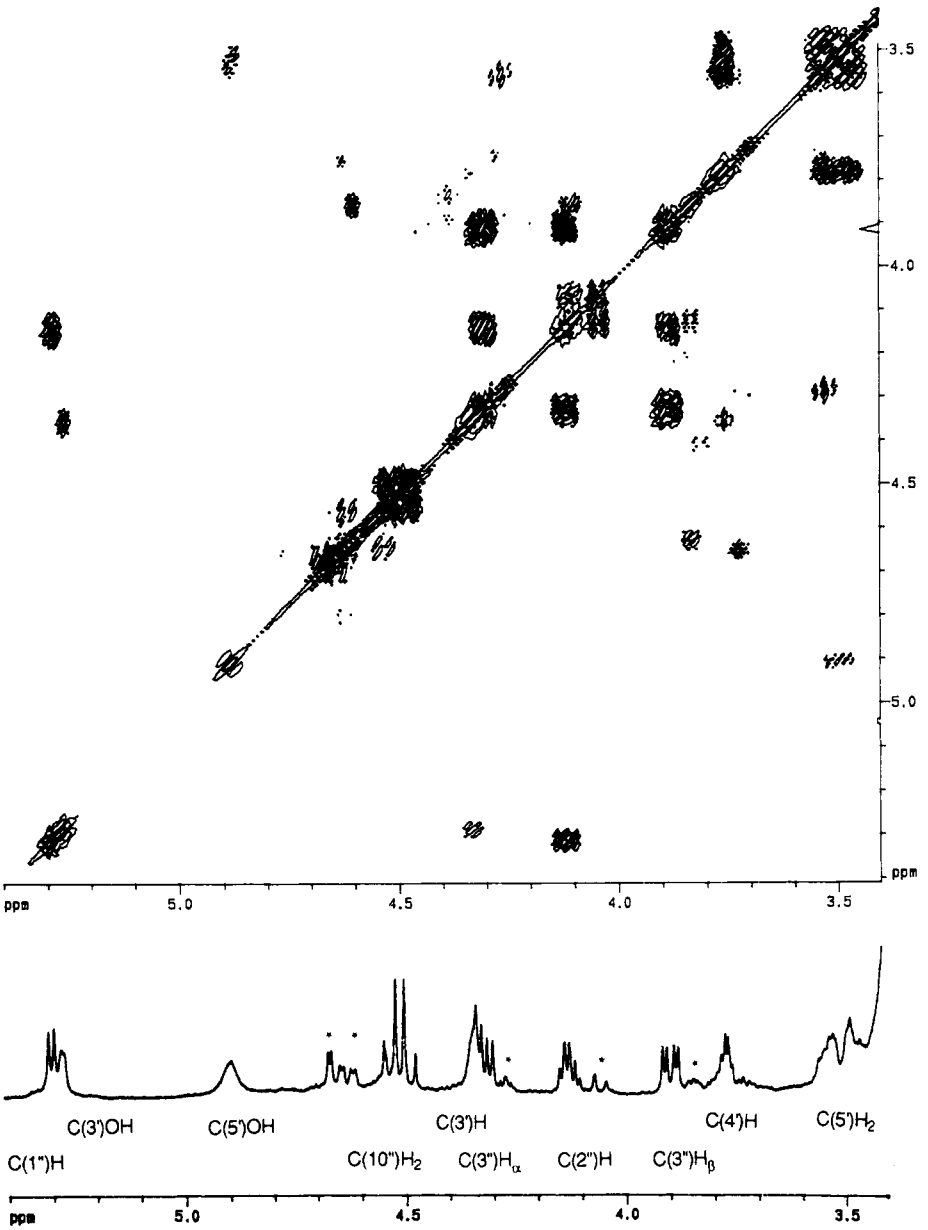

Figure 1. ^{1}H NMR and COSY spectra of δ 3.4–5.4 region of compound **3a** in DMSO-d_6. Impurity peaks are denoted by asterisks.

3b by mass spectrometry (+/− CI) were unsuccessful.

Conclusions

The present study coupled with our previous investigations[2i,j] demonstrates that $Cr(ClO_4)_2$-mediated reductive activation of **1** provides a simple and efficient procedure for the generation of C(1)- and C(10)-substituted mitosenes. Significantly, appreciable yields of C(10) nucleophilic substituted mitosene adducts are observed with this method in contrast with most other reductive activation techniques. This procedure has proven useful for the bonding of **1** to reagents of varying nucleophilic strength (i.e., ROH, 2′-deoxyguanosine, $PhNH_2$). We have attributed the efficiency of these nucleophilic substitution processes (in part) to the two one-electron reductions of the quinone ring in **1** to give a bis-Cr^{III}-bound mitomycin C complex. Formation of this species should permit mitomycin C(1) and C(10) nucleophilic reactions to proceed rapidly at the expense of the corresponding electrophilic transformations.[2i,j] The facility of the $Cr(ClO_4)_2$-induced mitomycin reactions suggests that this reductive activation method may find use for the selective modification of other biologically important quinone-containing compounds.

Experimental Section

General. HPLC analyses, pH measurements, and chromatographic procedures were performed as previously described.[1j] Solvent systems for chromatographies were A, methanol/chloroform (1:20), and B, methanol/chloroform (1:2) containing 0.2% triethylamine.

Procedure for the Reductive Activation of Mitomycin C (1a) with $Cr(ClO_4)_2$ in the Presence of Aniline. Preparation of Aniline-Substituted Compounds 2a and 2b. To a deaerated (Ar) MeOH solution (50 mL, "pH" 7.0) of **1a** (20 mg, 60 μmol) and aniline (5 equiv, 18 μL, 0.3 mmol) was added an aqueous solution of $Cr(ClO_4)_2$ (0.1 M, 1.2 mL, 2 equiv). The reaction solution was stirred at room temperature (1 h) under Ar, opened to the air, and evaporated in vacuo. The residue was chromatographed using preparative TLC (system A) to give a minor product **2a** (R_f 0.36, system A) and a major compound **2b** (R_f 0.42, system A).

10-Decarbamoyl-10-anilino-2,7-diaminomitosene (2a): HPLC retention time 32.4 min; ^{1}H NMR (DMSO-d_6) δ 1.71 (s, 3 H), 2.37 (dd, J = 5.1, 16.3 Hz, 1 H), 2.85 (dd, J = 7.1, 16.3 Hz, 1 H), 3.69 (dd, J = 4.8, 12.6 Hz, 1 H), 3.95–4.02 (m, 1 H), 4.18 (dd, J = 6.9, 12.6 Hz, 1 H), 4.27 (d, J = 5.7 Hz, 2 H), 5.86 (t, J = 5.7 Hz, 1 H), 6.47–6.54 (m, 3 H), 7.01–7.06 (m, 2 H); ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 3 H), 2.54 (dd, J = 4.5, 16.0 Hz, 1 H), 3.08 (dd, J = 6.9, 16.0 Hz, 1 H), 3.88 (dd, J = 4.3, 12.9 Hz, 1 H), 4.17–4.19

(m, 1 H), 4.33 (s, 2 H), 4.34 (dd, J = 6.4, 12.9 Hz, 1 H), 4.86 (br s, 2 H), 6.62 (d, J = 7.8 Hz, 2 H), 6.68 (t, J = 7.8 Hz, 1 H), 7.14 (t, J = 7.8 Hz, 2 H); the remaining NH signals were not identified; ^{13}C NMR (DMSO-d_6) δ 8.43, 33.27, 38.30, 54.74, 54.99, 104.14, 112.13, 115.09, 115.77, 121.07, 127.83, 128.81, 139.25, 146.79, 148.70, 176.48, 178.91; UV–vis (MeOH) λ_{max} 248, 311, 365 (sh), 540 nm.

***trans*-10-Decarbamoyl-1,10-dianilino-2,7-diaminomitosene (2b)**: HPLC retention time 36.2 min; ^{1}H NMR (DMSO-d_6) δ 1.74 (s, 3 H), 3.72–3.79 (m, 1 H), 3.86 (dd, J = 2.6, 12.8 Hz, 1 H), 4.18 (br s, 2 H), 4.30 (dd, J = 5.6, 12.8 Hz, 1 H), 4.46 (dd, J = 2.3, 7.4 Hz, 1 H), 5.42 (t, J = 5.6 Hz, 1 H), 5.94 (d, J = 7.4 Hz, 1 H), 6.45–7.15 (m, 10 H); ^{1}H NMR ($CDCl_3$) δ 1.84 (s, 3 H), 3.99 (dd, J = 3.1, 13.8 Hz, 1 H), 4.00–4.02 (m, 1 H), 4.39 (s, 2 H), 4.44 (dd, J = 6.6, 13.8 Hz, 1 H), 4.52 (d, J = 2.2 Hz, 1 H), 4.94 (br s, 2 H), 6.54–6.71 (m, 5 H), 6.83 (t, J = 7.8 Hz, 1 H), 7.09–7.27 (m, 4 H); the remaining NH signals were not identified; ^{13}C NMR (DMSO-d_6) δ 8.39, 38.67, 53.76, 58.20, 60.70, 104.42, 112.39, 112.91, 116.02, 116.09, 116.52, 121.50, 128.10, 128.70, 128.87, 139.56, 147.08, 147.56, 148.38, 176.49, 178.74; UV–vis (MeOH) λ_{max} 230, 252, 311, 365 (sh), 540 nm.

Procedure for the Reductive Activation of 10-Decarbamoylmitomycin C (1b) with $Cr(ClO_4)_2$ in the Presence of 2′-Deoxyguanosine. Decarbamoylmitomycin C (**1b**) (100 mg, 0.34 mmol) was added to a buffered aqueous solution (Tris·HCl (0.2 M), pH 8.15, 70 mL) containing 2′-deoxyguanosine monohydrate (290 mg, 1.70 mmol, 5 equiv), and the resulting suspension was heated at 40 °C until the reaction mixture became homogeneous (~10 min). The reaction was deaerated by passing a stream of Ar through the solution (20 min), and then $Cr(ClO_4)_2$ (0.1 M, 3.4 mL, 1 equiv) was added. The reaction was maintained under a positive pressure of Ar at room temperature (30 min), and then opened to the air. HPLC analysis of the reaction mixture indicated the presence of five major peaks. Three of these corresponded to the solvolytic products **2i–2k** and were verified by coinjection of authentic samples with the reaction mixture in the HPLC. The two other peaks eluted at 18.2 and 19.0 min. The solution was concentrated (~50 mL) in vacuo at ≤35 °C. The remaining solution was chromatographed on a Sephadex G-25 (fine) column (4.3 × 50 cm) using 0.02 M NH_4HCO_3 as the eluant to remove both excess 2′-deoxyguanosine and the chromium salts. The fractions containing the two HPLC peaks that eluted at 18.2 and 19.0 min were collected and lyophilized. The residue was dissolved in CH_3OH (5 mL) and chromatographed on preparative TLC plates using system B as the eluant to remove any remaining 2′-deoxyguanosine- and nondeoxyguanosine-bound mitosenes. The band at the origin was extracted with 10% aqueous CH_3OH and evaporated in vacuo. The residue was dissolved in H_2O (5 mL) and loaded on a Sephadex column (2.5 × 30 cm). Upon addition of the aqueous solution to the column a green-blue precipitate appeared at the top of the column. This precipitate did not move upon passage of the aqueous NH_4HCO_3 eluant through the column. Adduct **3a** eluted from the column, and then the Sephadex resin containing the precipitate was extracted with MeOH to give **3b**.

Compound 3b: ^{1}H NMR (DMSO-d_6, 500 MHz, 40 °C) δ 1.73 (s, C(6″)CH_3), 2.16–2.20 (m, C(2′)HH′), 2.52–2.57 (m, C(2′)HH′), 3.79 (br s), 3.81–3.82 (m, C(3″)H_β), 3.88 (br s), 4.32 (br s), 4.39–4.43 (m, C(3″)H_α), 4.51 (s, C(10″)H_2), 4.92 (br s, C(1″)H_β), 5.27 (br s), 6.16 (app t, J = 6.1 Hz, C(1′)H), 6.41 (br s, C(7″)NH_2), 7.83 (s, C(8)H).

Compound 3a: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.74 (s, C(6″)CH_3), 2.20–2.26 (m, C(2′)HH′), 2.52–2.58 (m, C(2′)HH′), 3.47–3.56 (m, C(5′)H_2), 3.77–3.79 (m, C(4′)H), 3.91 (dd, J = 4.7, 12.7 Hz, C(3″)H_β), 4.12–4.15 (m, C(2″)H), 4.33 (dd, J = 6.5, 12.7 Hz, C(3″)H_α), 4.35–4.37 (m, C(3′)H), 4.50 ($^1/_2$ ABq, J = 12.9 Hz, C(10″)HH′), 4.54 ($^1/_2$ ABq, J = 12.9 Hz, C(10″)HH′), 4.91 (br s, 1 H, C(5′)OH), 5.28 (br s, 1 H, C(3′)OH), 5.31 (d, J = 6.2 Hz, C(1″)H), 6.17 (app t, J = 6.8 Hz, C(1′)H), 6.51 (br s, C(7″)NH_2), 7.94 (s, C(8)H); the ^{1}H NMR assignments were supported by the corresponding COSY spectrum; ^{13}C NMR (DMSO-d_6, 75 MHz) δ 8.31 (C(6″)CH_3), 50.82 (C(3″)), 52.97 (C(2″)), 54.22 (C(10″)), 56.47 (C(1″)), 61.57 (C(5′)), 70.56 (C(3′)), 82.56 (C(4′)), 87.41 (C(1′)), 135.41 (C(8)); the C(2′) resonance is believed to be beneath the DMSO-d_6 peak; the other peaks were not detected; ^{13}C NMR (DMSO-d_6, 125 MHz) δ 8.94 (C(6″)CH_3), 50.59 (C(3″)), 52.71 (C(2″)), 53.87 (C(10″)), 56.08 (C(1″)), 61.09 (C(5′)), 69.91 (C(3′)), 81.68 (C(4′)), 86.47 (C(1′)), 103.17 (C(6″)), 115.56 (C(8a″)), 117.19 (C(5)), 119.17 (C(9″)), 126.22 (C(9a″)), 133.62 (C(7″) or C(8)), 135.96 (C(8) or C(7″)), 144.90 (C(5a″)), 150.09 (C(4)), 154.30 (C(2)), 173.86 (C(8″)), 175.97 (C(5″)). The C(2′) resonance was not observed as it is believed to be beneath the DMSO-d_6 peaks, and the C(6) signal was not detected.

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Supplementary Material Available: ^{1}H NMR spectra for compounds **2a**, **2b**, **3a**, and **3b**, ^{13}C NMR spectra for compounds **2a** and **3a**, and the APT NMR spectrum for compound **2b** (12 pages). Ordering information is given on any current masthead page.