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NEW SYNTHESIS AND BIOLOGICALLY ACTIVE MOLECULAR DESIGN OF DEAZAPTERIDINE-STEROID HYBRID COMPOUNDS

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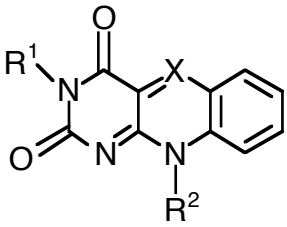
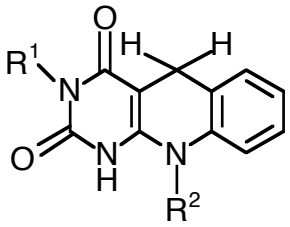
Abstract — This paper describes a facile and general synthesis of a new class of the hybrid compounds (**4**, **5** and **16**), possessing 5-deazapteridine and steroid in the same ring system, by condensation of 6-(monosubstituted amino)uracils (**9**) or 6-(monosubstituted amino)-2-phenylpyrimidin-4(3*H*)-ones (**14**) with 2-hydroxymethyleneandrostanolone (**10**) or 2-hydroxymethylenetestosterone (**15**) under heating in the presence of *p*-toluenesulfonic acid monohydrate and their potential unti-coccidiosis activities.

During the last three decade there has been considerable interest in synthesis, functional elucidation, and biological evaluation of 5-deazaflavins (5-deazaisoalloxazines) (**1a**) in which N-5 of the flavin (**1b**) is replaced by CH (**Scheme 1**). Actually, 5-deazaflavins (**1a**) have attracted great interest because of the first synthesis as potential flavin antagonists or flavin models,[1] the discovery that they serve as co-factors for several flavin catalyzed reactions,[2] and having the potent broad-spectrum activity against coccidiosis.[3] Moreover, it was found that the redox active coenzyme Factor 420 (F_{420}), which has 5-deazaflavin skeleton and absorbance maximum at 420 nm, was isolated from methanogenic bacteria.[4] Recently, 5-deazaflavins (**1a**) have been studied extensively in both enzymatic[2, 5] and model system.[6, 7] Above all, we have recently focused our interest on the study of the redox system, and we have reported the auto-recycling oxidation of alcohols catalyzed by 5-deazaflavins (**1a**)[7, 8] and their analogs[9-13] and the auto-recycling reduction of carbonyl compounds catalyzed by 1,5-dihydro-5-deazaflavins (**2**).[14] In addition, we found the selective PKC inhibitory activities of 5-deazaflavins (**1a**) and 2-deoxo-2-phenyl-5-deazaflavins (**3**), and reported their effective growth inhibition against cancer cells such as the A431 cells and HT1080 cells, which are known as the PKC concerning in the expression and proliferation.[15]

As a new trial in the active molecular design for drugs, we have most recently made the plan for synthesis of the hybrid compounds, which structurally contained two different biologically or pharmacologically active compounds like 5-deazaflavins (**1a**) and steroids, by expecting the bioactive potentiation or new bioactivities in the new hybrid compounds. We communicate here a novel and facile synthesis of the hybrid compounds (**4**, **5** and **16**) of 5-deazaflavins (5-deazapteridines) (**1a** and **3**) with steroids such as androstanolone (**6**) or testosterone (**7**).



1a : X = CH
b : X = N



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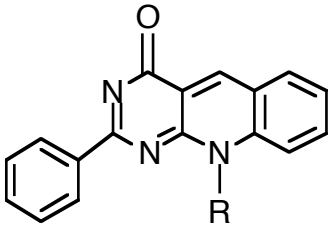
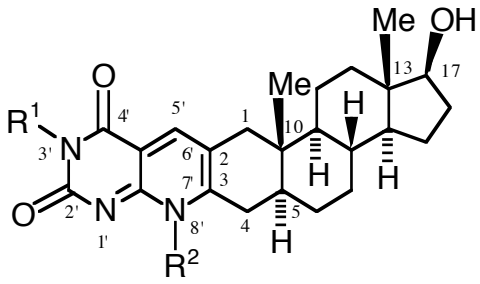


3

R^1 = H or Me
R, R^2 = Alkyl or Aryl



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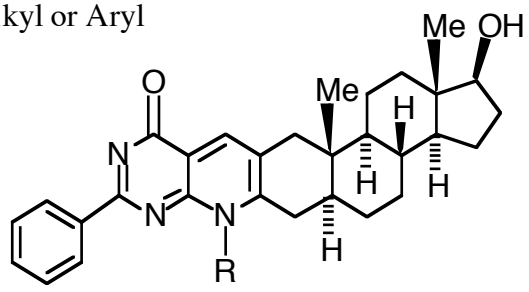

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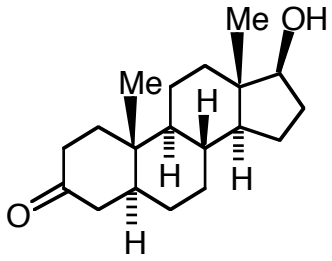

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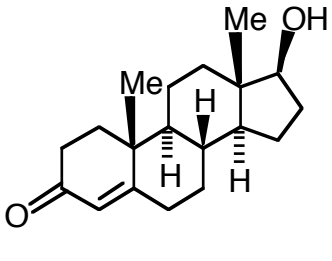

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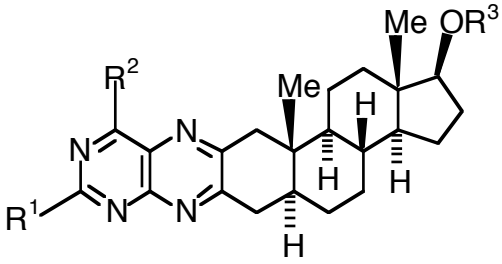
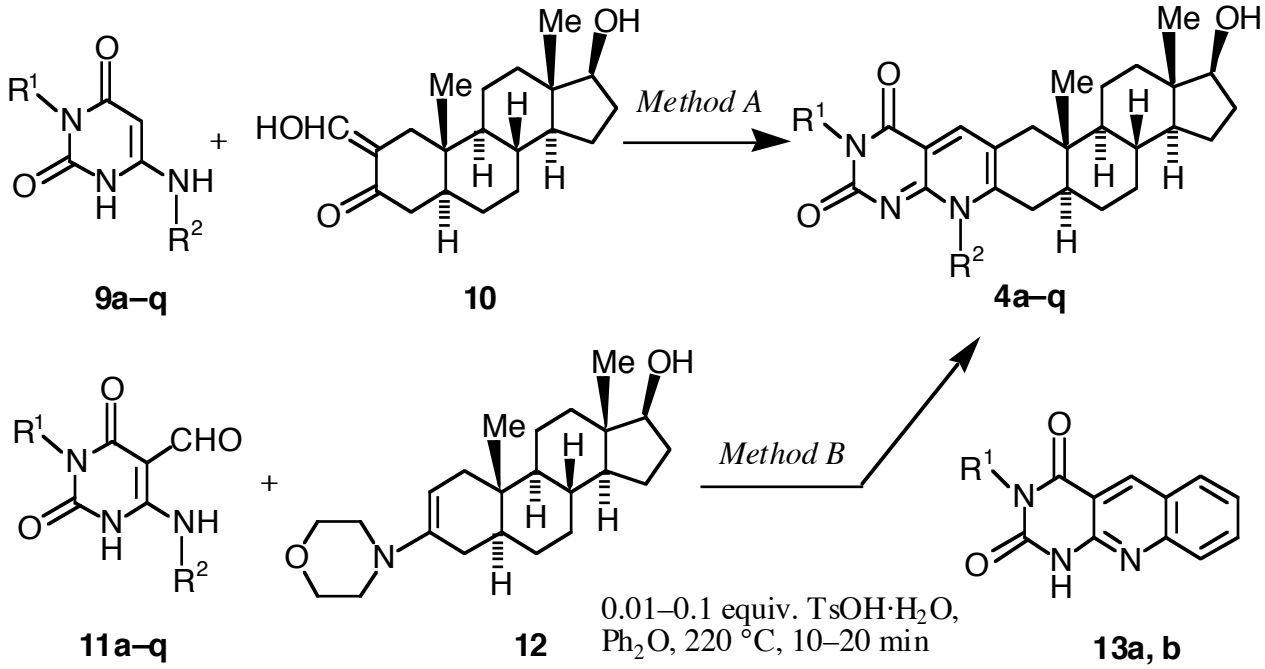


8

Scheme 1

The preparation of the hybrid compounds (**4** and **5**) possessing a 5'-deaza-5□-androst-2-eno[2,3-*g*]-pteridine ring system has not been reported hitherto. We now report the first synthesis of the hybrid compounds (**4**, **5** and **16**), which consists of condensation of 6-(monosubstituted amino)uracils (**9**) or 6-(monosubstituted amino)-2-phenylpyrimidin-4(3*H*)-ones (**14**) with 2-hydroxymethyleneandrostanolone (**10**) or 2-hydroxymethylenetestosterone (**15**) in the presence of *p*-toluenesulfonic acid monohydrate.



Scheme 2

Table 1. Preparation of 8'-substituted 5'-deaza-17β-hydroxy-5α-androst-2-eno[2,3-*g*]-pteridine-2',4'(3'*H*,8'*H*)-diones (**4a–q**)

Compd No.	R^1	R^2	Yield (%)		Mp (°C)[a]	$[\alpha]_D^{30}$ (°)[b]
			Method A	*Method B*		
4a	H	Me	60	40	255	*c*
4b	H	Et	77		320	*c*
4c	H	Pr	50	42	315	*c*
4d	H	Bn	52		297	*c*
4e	H	Ph	56	20	320	*c*
4f	H	4-MeO-C_6H_4	41	16	305	*c*
4g	H	4-Cl-C_6H_4	46	11	330	*c*
4h	Me	Me	82	61	228	+ 90
4i	Me	Et	86		322	+ 120
4j	Me	Pr	75	34	315	+ 104
4k	Me	Bu	83		311	+ 113
4l	Me	Bn	66		280	+ 174
4m	Me	Ph	64	14	330	+ 118
4n	Me	4-Me-C_6H_4	60		314	+ 107
4o	Me	4-MeO-C_6H_4	80	15	311	+ 87
4p	Me	4-F-C_6H_4	63		315	+ 105
4q	Me	4-Cl-C_6H_4	63	18	324	+ 99

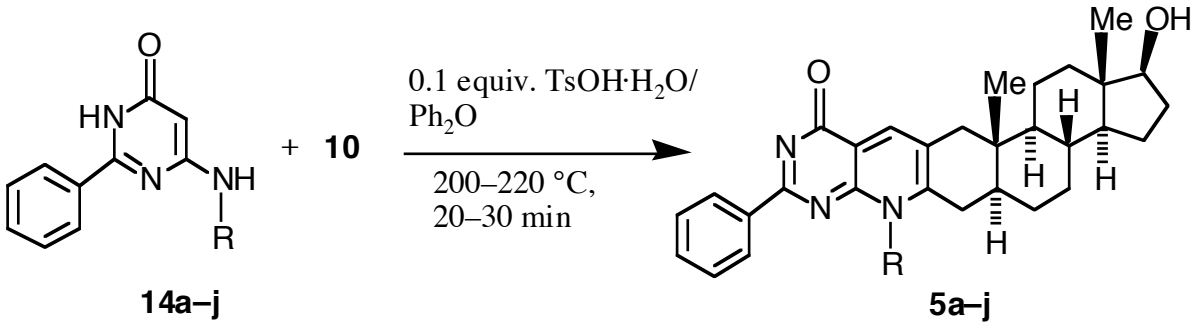
[a]All compounds were recrystallized from acetone or ethyl acetate and decomposed over those melting points. [b]All optical rotations were measured in chloroform (c = 2.1). [c]Since this compound did not dissolve sufficiently in any solvent at room temperature, the specific rotation was not obtained.

The key intermediate, 2-hydroxymethylene-17β-hydroxy1-5α-androstan-3-one (**10**), was prepared by treatment of androstanolone (**6**) with ethyl formate and sodium hydride according to the procedure described by Weisenborn *et al.*[16] Then, heating the intermediate (**6**) (3.54 mmol) with 6-methylamino-uracil (**9a**)[17] (3.54 mmol) and *p*-toluenesulfonic acid monohydrate (0.35 mmol) in diphenyl ether (1 mL) under an atmosphere of argon and stirring at 220 °C for 15 min, followed by subjecting to flash column chromatography on silica gel (Fuji Silysia 200–400 mesh; eluent: ethyl acetate), afforded the desired hybrid compound, 5'-deaza-17β-hydroxy-8'-methyl-5α-androst-2-eno[2,3-*g*]pteridine-2',4'(3'*H*,8'*H*)-dione (**4a**) in 60% yield (*Method A*). Other hybrid compounds (**4b–q**) were similarly prepared by heating **10** with appropriate 6-(monosubstituted amino)uracils (**9b–q**)[13, 18] and *p*-toluenesulfonic acid monohydrate (0.1 equiv.) in diphenyl ether under the same conditions in good yields as shown in **Scheme 2** and **Table 1**. All products (**4a–q**) exhibited satisfactory elemental combustion analyses and FAB-MS, IR, ^{1}H-NMR, ^{13}C-NMR and UV spectral data consistent with the structures. Interestingly, in the ^{1}H-NMR spectra in $CDCl_3$, the chemical shifts for protons at the 8'-positions of some compounds (**4b–d, i–l**) were remarkably affected by the asymmetric carbons on the steroid rings.[19] For example, two multiplet signals, which were attributable to the geminal protons of methylene of ethyl group at the 8'-position in the spectrum of compound (**4b**), were observed at δ 4.26–4.51 and δ 4.67– 4.92, respectively. In the case of compound (**4d**), the two doublet signals (J = 14.9 Hz) attributable to the geminal protons of methylene at the 8'-position were observed at δ 5.67 and δ 6.13, respectively.

In examining the previous reports on synthesis for analogous compounds of the hybrid compounds (**4** and **5**), we found a few reports[20–22] for the [2,3-*g*]-fused pteridinosteroids (**8**). It has been investigated in them that an enaminosteroid such as 3-morpholino-2-androstanolene for preparation of the pteridinosteroids (**8**) was useful. Therefore, we also applied an enaminosteroid (**12**) to the synthesis for the hybrid compounds (**4**). Thus, heating 17β-hydroxy-3-morpholino-5α-androst-2-ene (**12**)[23] (3.55 mmol) with appropriate 6-(monosubstituted amino)-5-formyluracils[24, 25] (3.55 mmol) and *p*-toluenesulfonic acid monohydrate (0.035 mmol) in diphenyl ether (1 mL) under an atmosphere of argon and stirring at 220 °C for 10–20 min, followed by subjecting to flash column chromatography as noted above, yielded the corresponding hybrid compounds (**4**) in lower yields (*Method B*). Especially, the poor yields (*ca.* 10– 20%) in the case of the condensation of **12** with 6-arylamino-5-formyluracils (**11e, f, g, m, o, q**) were observed because of instability of the enaminosteroid (**12**) in high temperature and formation of pyrimido[4,5-*b*]quinoline-2,4(1*H*,3*H*)-diones (**13a**: R^1 = H and **13b**: R^1 = Me),[26, 27] which were actually isolated as by-products, depend on cyclization of the 6-arylamino derivatives (**11e, m**).

The synthesis of 8'-substituted 5'-deaza-17β-hydroxy-2'-phenyl-5α-androst-2-eno[2,3-*g*]pteridine-4'(8'*H*)-ones (**5a–j**) was accomplished in a similar manner as above. Thus, a mixture of 2-hydroxy-

methyleneandrostanolone (**10**) (2.85 mmol) with appropriate 6-(monosubstituted amino)-2-phenyl-pyrimidin-4(3*H*)-ones (**14**)[13, 28] (2.85 mmol) and *p*-toluenesulfonic acid monohydrate (0.28 mmol) in diphenyl ether (0.5 mL) under an atmosphere of argon was heated at 200–220 °C for 20–30 min with stirring to give the corresponding hybrid compounds (**5a–j**), which were isolated by subjecting to flash column chromatography on silica gel (Fuji Silysia 200–400 mesh; eluent: ethyl acetate/ethanol, 9:1) and were recrystallized from ethyl acetate or acetone, in good yields (**Scheme 3** and **Table 2**). The asymmetric carbons also affected the chemical shifts for protons at the 8'-positions of some compounds (**5b–d**) in the ^{1}H-NMR spectra.[19]



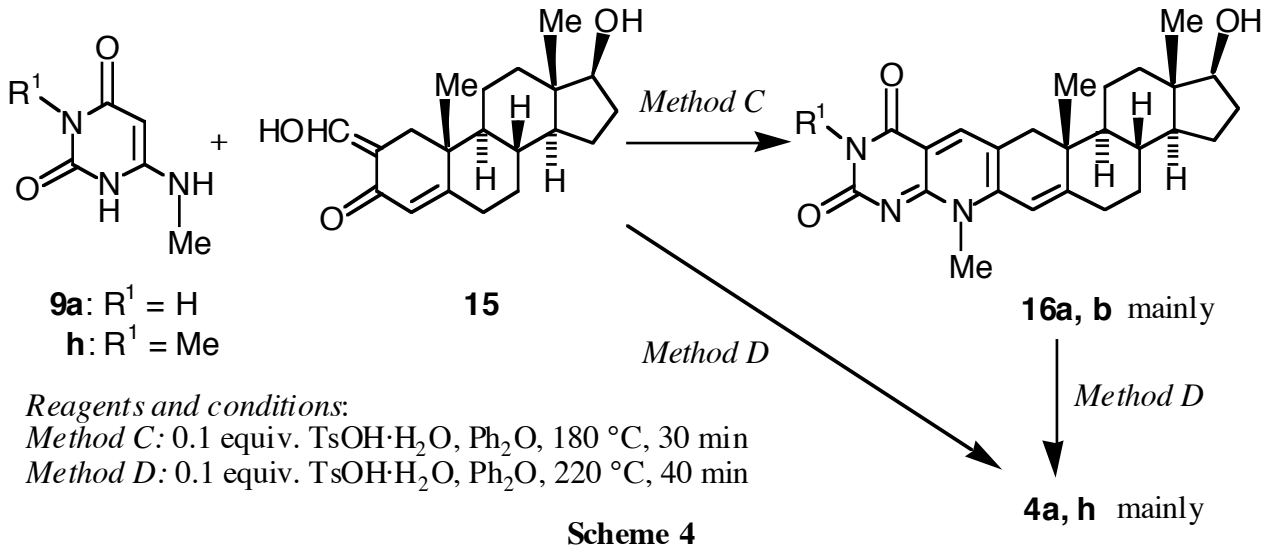
Scheme 3

Table 2. Preparation of 8'-substituted 5'-deaza-17β-hydroxy-2'-phenyl-5α-androst-2-eno[2,3-*g*]pteridin-4'(8'*H*)-ones (**5a–j**)

Compd No.	R	Yield (%)	Mp (°C)[a]	Compd No.	R	Yield (%)	Mp (°C)[a]
5a	Me	76	235	**5f**	cyclohexyl	60	230
5b	Et	72	243	**5g**	Ph	40	230
5c	Pr	73	215	**5h**	2,6-Me_2-C_6H_3	48	235
5d	Bu	75	215	**5i**	4-MeO-C_6H_4	45	240
5e	Bn	65	245	**5j**	4-Cl-C_6H_4	43	250

[a]All compounds were recrystallized from acetone or ethyl acetate and decomposed over those melting points.

In addition, we tried to prepare the hybrid compounds (**16a, b**) by condensation of 6-methylaminouracils (**9a, h**) with 2-hydroxymethylenetestosterone (**15**)[29] under similar conditions described above (**Scheme 4**). That is, heating compound (**15**) with 6-methylaminouracil (**9a**) (equiv.) and *p*-toluenesulfonic acid monohydrate (0.1 equiv.) in diphenyl ether under stirring at 180 °C for 30 min, followed by subjecting to flash column chromatography, afforded the desired hybrid compound, 5'-deaza-17β-hydroxy-8'-methylandrost-2,4-dieno[2,3-*g*]pteridine-2',4'(3'*H*,8'*H*)-dione (**16a**, mp 255 °C, decomp), and the reduced compound (**4a**) in 37% and 9% yield, respectively. The same reaction of **15** with 3-mehtyl-6-



Scheme 4

methylaminouracil (**9h**) gave the desired 3'-methyl derivative (**16b**, mp 230 °C, decomp, 43%) and the reduced compound (**4h**, 7%) (*Method C*). On the other hand, the similar reaction of **15** with **9a** and *p*-toluenesulfonic acid monohydrate in diphenyl ether under stirring at 220 °C for 40 min gave the corresponding **16a** and **4a** in 6% and 32% yields, respectively, and an unidentified compound. The same reaction of **15** with **9h** gave the corresponding **16b** (21%), **4h** (23%), and an unidentified compound (*Method D*). The reduced compounds (**4a, h**) were identical with those obtained by *Method A*. It is interesting to note that the hybrid compounds (**16**) derived from testosterone (**7**) were converted to the corresponding hybrid compounds (**4**) derived from androstanolone (**6**) by redox reaction under the above conditions with high temperature. In fact, heating compounds (**16a, b**) with *p*-toluenesulfonic acid monohydrate in diphenyl ether afforded the corresponding reduced compounds (**4a, h**) in *ca*. 50% yield and unidentified compounds (*Method D*). The reaction mechanism on redox system for the condensation reaction is now under investigation.

Some of them showed more potent unti-coccidiosis activities than that of robenidine *in vitro*.[30]

Thus, the reliable and general synthetic method is noteworthy owing to the availability of such hybrid compounds containing many kinds of pyrimidines, pyridines, flavin, deazaflavin and steroids. Further synthetic and mechanistic investigations and biological activities of the hybrid compounds (**4**, **5** and **16**) are in progress, and will be reported in detail shortly.

ACKNOWLEDGEMENTS

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19. *^{1}H- NMR spectral data in $CDCl_3$*. For **4b**: 0.78 (6H, s, 18 and 19-CH_3), 1.44 (3H, t, *J* 6.9 Hz, 8'-CH_2CH_3), 2.45 (1H, d, *J* 16.1 Hz, 1□-H), 2.57 (1H, dd, *J* 18.1, 10.2 Hz, 4□-H), 2.76 (1H, d *J* 16.1 Hz, 1□-H), 2.88 (1H, dd, *J* 18.1, 4.4 Hz, 4□-H), 3.67 (1H, dd, $J_{16□,17□}$ 8.4, $J_{16□,17□}$ 8.2 Hz, 17□-H), 4.26–4.51 (1H, m, 8'-CH_aH$_b$), 4.67–4.92 (1H, m, 8'- CH$_a$$H_b$), 8.26 (1H, s, 5'-H), 8.37 (1H, s, 3'-NH). For **4c**: 0.78 (6H, s, 18 and 19-CH_3), 1.06 (3H, t, *J* 7.4 Hz, 8'-$CH_2CH_2CH_3$), 2.45 (1H, d, *J* 16.3 Hz, 1□-H), 2.54 (1H, dd, *J* 18.1, 11.0 Hz, 4□-H), 2.77 (1H, d *J* 16.3 Hz, 1□-H), 2.85 (1H, dd, *J* 18.1, 4.6 Hz, 4□-H), 3.68 (1H, dd, $J_{16□,17□}$ 8.4, $J_{16□,17□}$ 8.2 Hz, 17□-H), 4.12–4.38 (1H, m, 8'-CH_aH$_b$),

4.54–4.76 (1H, m, 8'- CH_aH_b), 8.26 (1H, s, 5'-H), 8.34 (1H, s, 3'-NH). For **4d**: 0.71 (3H, s, 18-CH_3), 0.75 (3H, s, 19-CH_3), 2.32–2.52 (1H, m, 4□-H), 2.44 (1H, d, *J* 16.0 Hz, 1□-H), 2.68–2.88 (1H, m, 4□-H), 2.76 (1H, d *J* 16.0 Hz, 1□-H), 3.65 (1H, dd, $J_{16□,17□}$ 8.4, $J_{16□,17□}$ 7.8 Hz, 17□-H), 5.67 (1H, br d, *J* 14.9 Hz, 8'-CH_aH_b), 6.13 (1H, br d, *J* 14.9 Hz, 8'-CH_aH_b), 6.96–7.13 (2H, m, Ph-*m*-H), 7.22–7.37 (3H, m, Ph-*o, p*-H), 8.34 (1H, s, 5'-H), 8.44 (1H, s, 3'-NH). For **5b**: 0.79 (6H, s, 18 and 19-CH_3), 1.54 (3H, t, *J* 7.0 Hz, 8'-CH_2CH_3), 2.57 (1H, d, *J* 16.7 Hz, 1□-H), 2.63 (1H, dd, *J* 19.6, 11.2 Hz, 4□-H), 2.89 (1H, d *J* 16.7 Hz, 1□-H), 3.00 (1H, dd, *J* 19.6, 5.2 Hz, 4□-H), 3.70 (1H, dd, $J_{16□,17□}$ 8.8, $J_{16□,17□}$ 8.0 Hz, 17□-H), 4.62–4.79 (1H, m, 8'-CH_aH_b), 4.90–5.07 (1H, m, 8'- CH_aH_b), 7.40–7.52 (3H, m, Ph-*m, p*-H), 8.53–8.65 (2H, m, Ph-*o*-H), 8.62 (1H, s, 5'-H). For **5c**: 0.79 (6H, s, 18 and 19-CH_3), 1.15 (3H, t, *J* 7.3 Hz, 8'-$CH_2CH_2CH_3$), 2.57 (1H, d, *J* 16.2 Hz, 1□-H), 2.64 (1H, dd, *J* 18.8, 10.2 Hz, 4□-H), 2.91 (1H, d *J* 16.2 Hz, 1□-H), 2.98 (1H, dd, *J* 18.8, 5.2 Hz, 4□-H), 3.69 (1H, dd, $J_{16□,17□}$ 8.0, $J_{16□,17□}$ 7.8 Hz, 17□-H), 4.45–4.68 (1H, m, 8'-CH_aH_b), 4.77–4.98 (1H, m, 8'- CH_aH_b), 7.40–7.53 (3H, m, Ph-*m, p*-H), 8.54–8.65 (2H, m, Ph-*o*-H), 8.67 (1H, s, 5'-H). For **5d**: 0.79 (6H, s, 18 and 19-CH_3), 1.06 (3H, t, *J* 7.2 Hz, 8'-CH_2CH_2 CH_2CH_3), 2.58 (1H, d, *J* 16.0 Hz, 1□-H), 2.63 (1H, dd, *J* 20.0, 11.3 Hz, 4□-H), 2.91 (1H, d *J* 16.0 Hz, 1□-H), 3.00 (1H, dd, *J* 20.0, 5.0 Hz, 4□-H), 3.69 (1H, dd, $J_{16□,17□}$ 8.0, $J_{16□,17□}$ 7.2 Hz, 17□-H), 4.47–4.70 (1H, m, 8'-CH_aH_b), 4.80–5.03 (1H, m, 8'- CH_aH_b), 7.40–7.55 (3H, m, Ph-*m, p*-H), 8.53–8.63 (2H, m, Ph-*o*-H), 8.65 (1H, s, 5'-H).

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