# *Communications*

## Synthesis of the PhIP Adduct of 2′-Deoxyguanosine and Its Incorporation into Oligomeric DNA

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The 2′-deoxyguanosine adduct of the dietary mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) has been synthesized and incorporated into DNA using solid state synthesis technology. The key step to obtaining the C8-dG adduct is a palladium (Xantphos-chelated)-catalyzed *N*-arylation (Buchwald−Hartwig reaction) of PhIP by a suitably protected 8-bromo-2′-deoxyguanosine derivative. The reaction proceeded in good yield without complicating side products, and the adduct was converted to the required 5′-O-DMT-3′-O-phosphoramidite by standard methods. This modified deoxynucleoside was used to synthesize three oligodeoxynucleotides in which the C8-PhIP-dG adduct was incorporated at a single site. The oligomers were purified by reverse phase HPLC and characterized by mass spectrometry.

### Introduction

A considerable number of polycyclic aromatic amines (PAAs)[1] are now well-established as precursors to mutagenic species that are strongly implicated as some of the causative factors in mammalian cancer (*1*). The amines themselves have little physiological activity, but by well-understood hepatic metabolic processes, they are first converted by microsomal enzymes to the *N*-hydroxy derivatives, which in turn undergo transformation to the *O*-acetyl or *O*-sulfonate esters by conjugating transferases. These esters, on solvolysis, give rise to highly reactive electrophilic nitrenium and/or isomeric carbocations (*2*), which are the true promutagens in that they react with DNA, mainly with the purine bases, to give stable adducts. In general, the nitrenium ions attack these bases at the C8 position, whereas the carbocations are largely restricted to reaction at the exocyclic amino groups.

The recently discovered (*1*) dietary mutagens also fall into the carcinogenic amine class, being polycyclic heteroaromatic amines in nature. Although a wide array of premutagens is found in the human diet, of great interest currently are the extremely potent precursor carcinogens 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) (**1**), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) (**2**), and 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) (**3**), all members of the condensed 2-aminoimidazole class of premutagens (Figure 1). These particular substances are thought to arise (*1*) from the condensation of amino acids with creatinine, when proteinaceous food such as meat, fish, and poultry is cooked above 120 °C. Interestingly, they have also been found in cigarette smoke and in rainwater

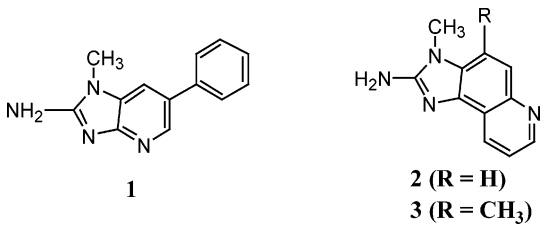


**Figure 1.** Chemical structures of the food mutagens **1**−**3**.

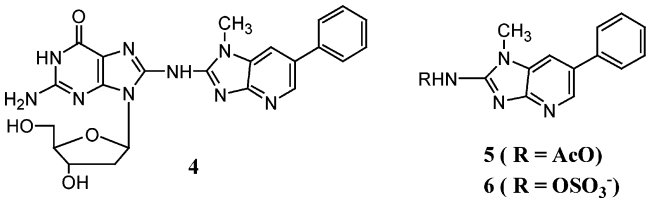


**Figure 2.** Chemical structures of the C8-dG adduct of PhIP **4** and two reactive PhIP metabolites **5** and **6**.

(*3*, *4*). Because of the presence of PhIP in cigarette smoke condensate (*5*), it is not surprising that this amine is a major mutagenic component recovered from the urine of tobacco smokers (*6*). It has been estimated that the average American citizen is exposed daily to PhIP, at the level of 16−200 ng/kg (*6*). The metabolic transformations of these substances are identical to those of the PAAs; however, the mutagenicities associated with the metabolites of these byproducts of cooking appear, on a molar basis, to be substantially greater (*1*) than those derived from the simpler carcinogenic amines.

2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine or PhIP (**1**) continues to receive significant attention because not only is it the most mass abundant of the dietary mutagens that occur in cooked meat and fish, but in mammals, it is one of the most potent colon and breast carcinogens isolated to date (*7*). Interest in this compound has been further heightened by the identification of PhIP adducts in colon tissue that was removed from

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[1]Abbreviations: BH, Buchwald−Hartwig reaction; DMT, dimethoxytrityl; ESI, electrospray ionization; IQ, 2-amino-3-methylimidazo[4,5-f]-quinoline; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline; PAA, polycyclic aromatic amine; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

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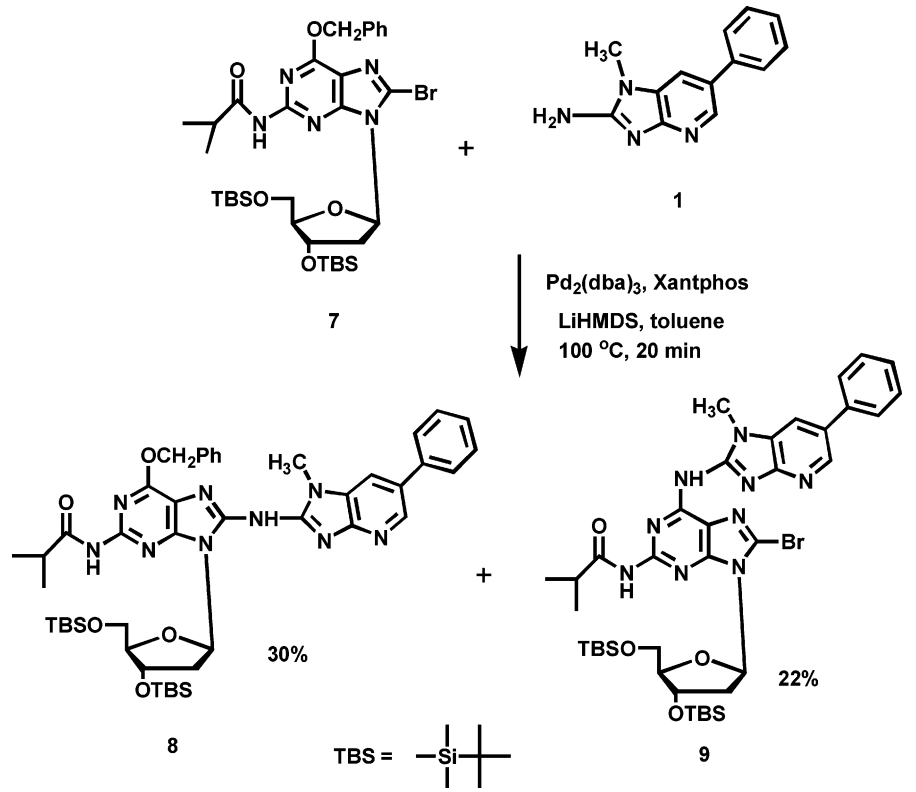
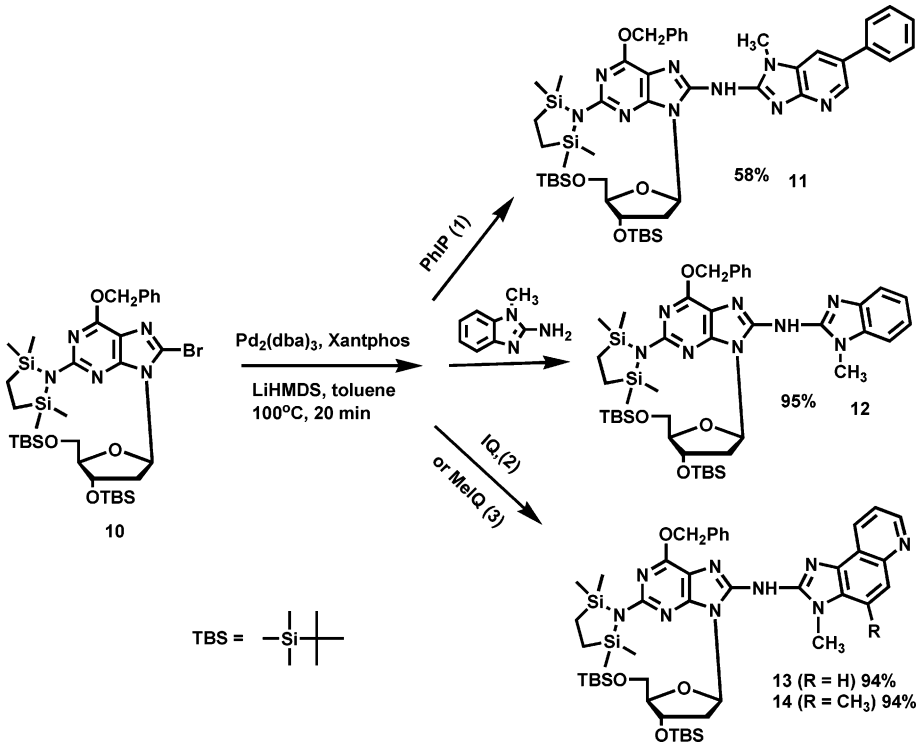
**Scheme 1**



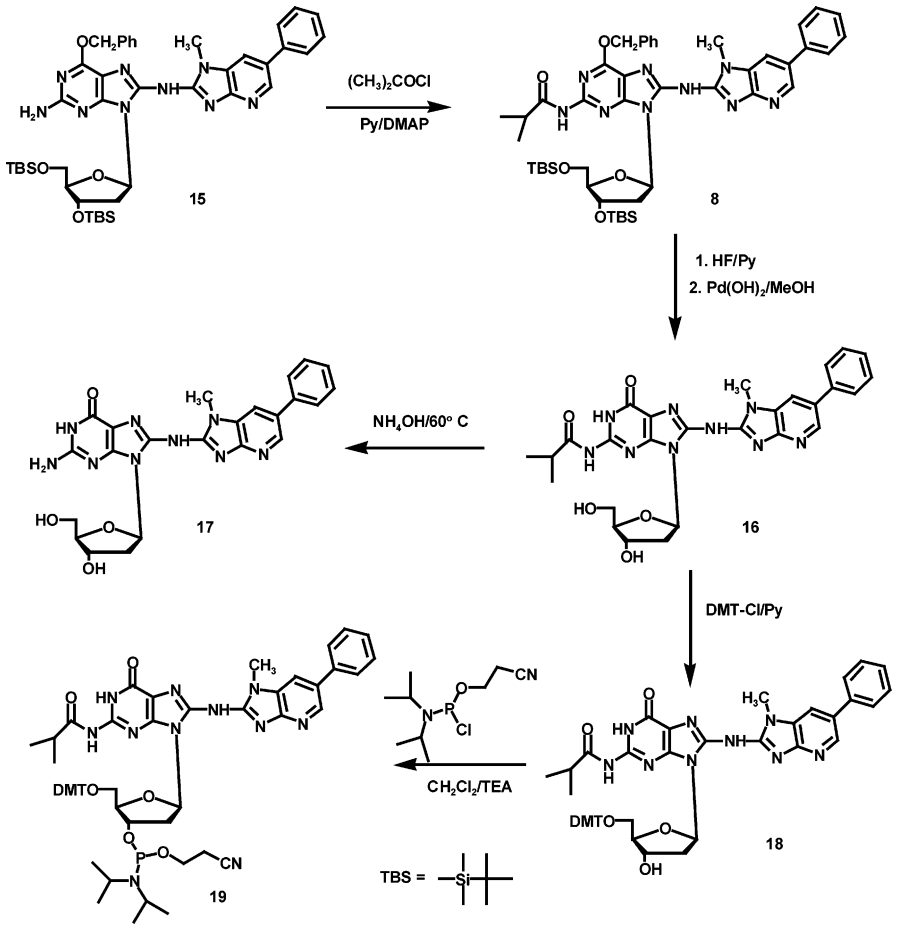

**Scheme 2**




adult humans undergoing surgery as part of their therapy for colon cancer (*8*). The principal adduct is the deoxyguanosine derivative **4** derived from the metabolite (**5** or **6**) of PhIP (Figure 2). Adduct **4** was also confirmed as the major DNA lesion in the tissues of rats that were treated with PhIP, using $^{32}$P-postlabeling analysis (*7*).

Our interest in PhIP and its dG adduct arose because methods of synthesis of the latter substance were limited to the acid-catalyzed solvolysis of the *N*-acetoxy derivative **5** in the presence of 2′-deoxyguanosine (*9*). However, yields of **4** by this method are exceedingly low, and to permit more extensive biological and physicochemical studies of this adduct, a much better synthetic approach was required. We would now like to report a significantly more efficient procedure for the synthesis of **4** and its incorporation into oligomeric DNA using standard automated methods (*10*).

**Scheme 3**




## Results and Discussion

Our approach is similar to that used for the synthesis and incorporation of the benzo[*a*]pyrene adducts into DNA in which the key procedure is a Buchwald−Hartwig (BH) reaction (*11*). Initial attempts to use the standard BH coupling of the protected form of 8-bromo-dG (**7**) with PhIP using the conditions outlined in Scheme 1 did not proceed well. Although the desired coupled product **8** was obtained in 30% yield, it was contaminated with 22% of the unusual substance **9**, in which we have assigned a PhIP residue at C6 in place of the benzyloxy function and the bromine atom is retained at C8. Separation of these substances was not difficult by column chromatography, but the occurrence of **9** was an unwanted complication. This difficulty was resolved by changing the 2-amino protecting group on the deoxyguanosine from isobutyryl to the tetramethyl-bis-silylethyl group used by Wang and Rizzo (*12*) in their synthesis of the dG adduct of IQ. Thus, when **10** was used in the coupling procedure (Scheme 2) with PhIP employing 4,5-bis(diphenylphosphino)-9,9-dimethyl xanthene (Xantphos) as the Pd ligand under the conditions described by Yin et al. (*13*), the required bis-heteroarylamine **11** was obtained in 58% yield (after chromatography) with virtually no significant contamination by the analogue corresponding to **9** or by other coupled products[2] (*14*). Importantly, this procedure proved to be highly successful when applied to the coupling reaction of **10** with a series of other related 2-aminoimidazoles. 1-Methyl-2-aminobenzimidazole led to the adduct **12** in 95% yield, whereas IQ and MeIQ gave adducts **13** and **14**, respectively, both in 94% yield. This yield is considerably better than that reported (*12*) previously for the coupling reaction of IQ with a similarly protected dG intermediate but in which BINAP was used as the Pd ligand rather than Xantphos.

**Table 1**

| oligomer sequence X = C8-PhIP-dG residue | calculated mass (Da) | observed average mass (Da) |
|---|---|---|
| a. 5′-TT X TTT-3′ | 2010.2 | 2011.2 ± 0.07 |
| b. 5′-CTC CTC XAT ACC T-3′ | 4067.6 | 4068.8 ± 0.33 |
| c. 5′-TTC CCT CCT CXA TAC CT-3′ | 5254.4 | 5256.5 ± 0.42 |

The bis-silyl protecting group on the 2-amino group of **11** was immediately removed by mild acid hydrolysis to give **15**, which was then treated with isobutyryl chloride to give the more stable **8**. The resulting product **8** (Scheme 3) was then desilylated by means of HF in anhydrous pyridine, and the benzyl group on the six-oxygen atom of the deoxyguanosine was removed by hydrogenation over a Pd catalyst to give **16** (*15*) in an overall yield of 80%. Compound **16** was heated in $NH_4OH$ at 60 °C to remove the isobutyryl group yielding the xenodeoxynucleoside **17**, which was identical to the PhIP-dG adduct reported in the literature (*16*). Conversion of **16** in two steps (60%) to the dimethoxytrityl (DMT) phosphoramidite **19**, suitable for incorporation into DNA, was accomplished via **18** using conventional methods.

Compound **19** was then utilized in the automated synthesis (*17*) of the three oligomers listed in Table 1 in which X represents the C8-PhIP-dG residue.

[2]After this work was completed, Dr. Takeji Takamura of the National Cancer Center Research Institute, Tokyo, Japan, informed us privately that he also had been able to synthesize **4** by means of a BH reaction using different protecting groups on the 2′-deoxyguanosine moiety.

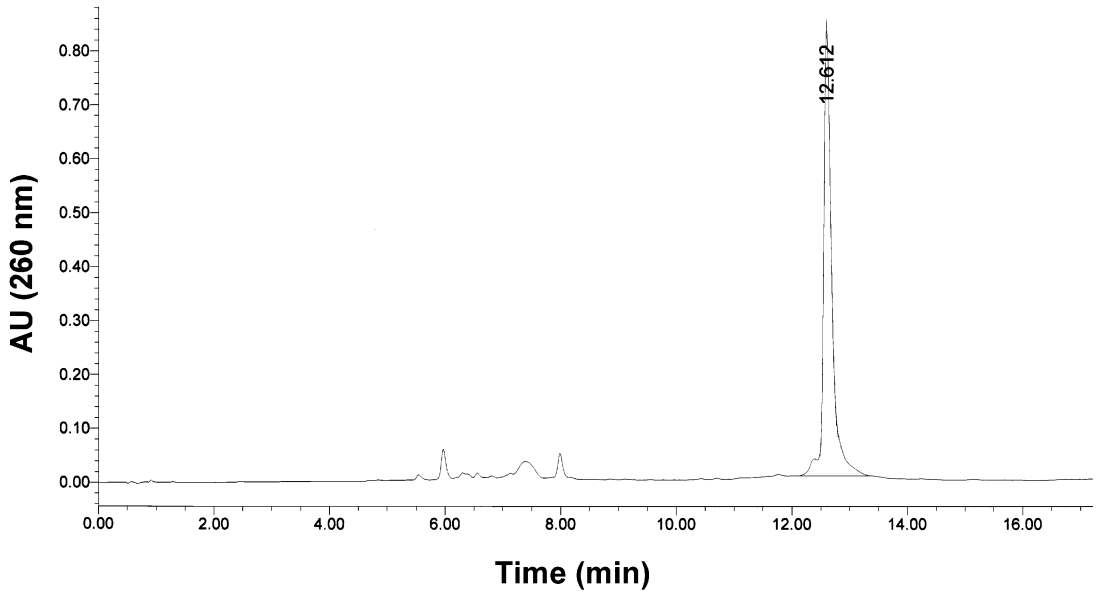



**Figure 3.** Final HPLC chromatogram for the fully deprotected oligomer b (DMT-off).

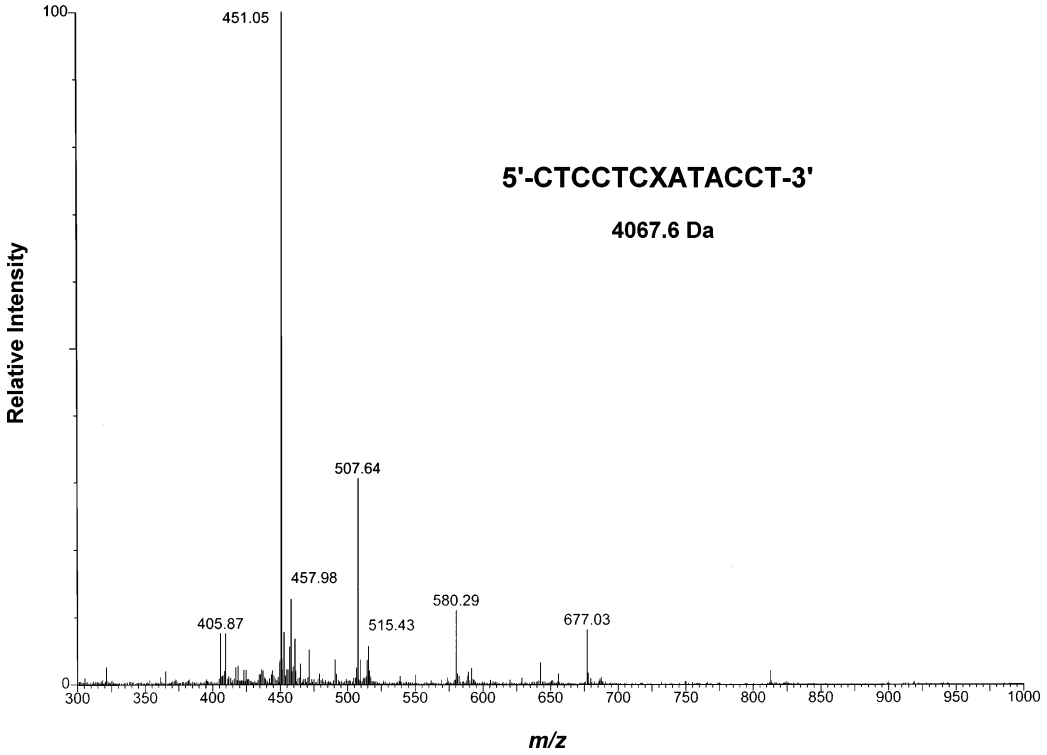



**Figure 4.** Electrospray mass spectrum for oligomer b yielding an average mass of 4068.8 ± 0.3 Da (calcd mass, 4067.6 Da).

The coupling yield was 78−80% for the small oligomer when a reaction time of 15 min was used at the point of introduction of the xenonucleoside; increasing this time to 30 min effected no improvement in yield. However, under similar conditions, the coupling yields for the synthesis of the larger oligomers were reduced to approximately 40%. Deprotection of the oligomers was accomplished first by means of 28% $NH_3$/water at 50 °C in a sealed tube yielding the DMT-protected form of the oligomers. Purification was accomplished by two consecutive HPLC passes under conditions reported elsewhere (*11*, *18*). In the first pass, the DMT-protected oligomer was separated from the failure sequences lacking DMT residues and collected as a pure fraction. This fraction was dried, and the DMT group was removed by treatment with 80% acetic acid in water at room temperature. Thereafter, the oligomers (DMT-off) were again subjected to final HPLC purification (Figure 3, oligomer b). Removal of the solvent under vacuum afforded the dry, purified oligomers, which are stable for at least 6 months when stored at −20 °C. The molecular weights of the oligomers were confirmed by electrospray ionization (ESI) mass spectrometry (Table 1) using a Waters Platform LC-MS system. A typical mass spectrum (oligomer b) is shown in Figure 4. The use of these oligomers for physicochemical and mutagenic studies will be reported in separate publications.

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(14) An oven-dried 50 mL two-necked round-bottomed flask was charged with fully protected 8-Br-dG (**10**, 603.75 mg, 0.75 mmol), tris-(dibenzylidene-acetone)dipalladium (27.45 mg, 0.03 mmol), 4,5-bis-(diphenylphosphino)-9,9-dimethyl-xanthene (xantphos, 18.9 mg, 0.033 mmol), and PhIP (**1**, 235.2 mg, 1.05 mmol) in dioxane under nitrogen. A solution of LiHMDS (1 M solution in THF, 1.5 mL, 1.5 mmol) was added, and the reaction mixture was stirred for 30 min at room temperature and then heated at 100 °C for 1 h. The reaction mixture was diluted with methylene chloride (20 mL) and aqueous saturated $NaHCO_3$ (10 mL). The organic layer was separated and extracted aqueous layer with methylene chloride (10 mL), and the combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude residue was purified by column chromatography using methylene chloride as eluant (TEA coated silica gel used) to give pure coupled product **11** as a colorless solid (412 mg, 58%). $^1$H NMR ($CDCl_3$): $\delta$ 8.46 (s, 1H), 7.66 (d, 2H), 7.48 (m, 10H), 6.78 (m, 1H), 5.62 (s, 2H), 4.64 (m, 1H), 4.06 (m, 1H), 3.92 (m, 3H), 3.76 (s, 3H), 2.12 (m, 1H), 1.04 (s, 9H), 0.98 (s, 9H), 0.91 (s, 4H), 0.44 (s, 12H), 0.23 (s, 6H), 0.13 (s, 6H). FAB-MS *m*/*z* 950 $[M + 1]^+$. A similar procedure was used to couple IQ, MeIQ, and *N*-methyl benzimidazole.

(15) $^1$H NMR ($CDCl_3$ + drop of $CD_3OD$): $\delta$ 8.09 (s, 1H), 7.23−7.73 (m, 7H), 6.98 (t, 1H), 5.01 (m, 1H), 4.04 (m, 1H), 3.92 (m, 2H), 3.65 (s, 3H), 3.12 (m, 1H), 2.89 (m, 1H), 2.41 (m, 1H), 1.31 (s, 3H), 1.29 (s, 3H). FAB-MS *m*/*z* 560 $[M + 1]^+$.

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