aliquot was placed in a hemocytometer chamber, and the number of cells/cm^3 was calculated. Then, 10^6 cells were injected ip into each test animal using an 18-gauge needle. 6-Mercaptopurine and melphalan were used as internal standards in the test. Test drugs were homogenized in 1% carboxymethylcellulose and administered ip at 20 (mg/kg)/day for 9 days. After 10 days, the inoculated mice were sacrificed, and the ascitic fluid was collected. The volume (mL) of the ascitic fluid was measured, and the total packed ascites cell volume for each group was measured utilizing nonheparinized capillary tubes centrifuged at 3000 rpm for 3–5 min. The control (Tween 80) (C) value for the volume of tumor was 10.62 ± 0.48 (SD) mL and for the ascrit (total packed cell volume) was 32.4 ± 1.69 mL at day 10. Percent inhibition of tumor growth was calculated by the following formula for the treated animals (T):

$$\% \text{ inhibn} = 100 - \left(\frac{\text{vol}_T \times \text{ascrit}_T}{\text{vol}_C \times \text{ascrit}_C}\right)100$$

Any compound that exhibited 80% inhibition of tumor growth was considered significantly active.

Lymphocytic Leukemia P-388 Screen (Table IV). The lymphocytic leukemia P-388 test was carried out in DBA/2 male mice (20 g). In this screen, 10^6 cells were implanted on day 0. The test compounds were administered ip at 20 (mg/kg)/day for 2 weeks. T/C values were calculated according to the NIH protocol.[21] 5-Fluorouracil was used as the internal standard in this test.

Acknowledgment. This research was sponsored by a grant from the Research Committee of Ferdowsi to Z.S. and a grant (VC785) from the Faculty Research Council at the University of North Carolina to I.H.H.

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Synthesis and Mutagenicity of A-Ring Reduced Analogues of 7,12-Dimethylbenz[*a*]anthracene[1,2]

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The synthesis and mutagenicity of two derivatives of 7,12-dimethylbenz[*a*]anthracene (DMBA; 1), i.e., 1,2-H_2DMBA (4) and 1,2,3,4-H_4DMBA (5), are reported. These analogues (4 and 5) represent dihydro and tetrahydro A-ring reduced forms of DMBA, a region in the parent hydrocarbon (1) proposed to be involved in metabolism to the ultimate carcinogen. The synthesis for 4 without isolation of intermediates from the tosylhydrazone of 1,2,3,4-tetrahydrobenz[*a*]anthracene-4,7,12-trione (10) by successive reaction with 8 molar equiv of CH_3Li, HI, and $NaBH_4$ represents a novel approach to this hydrocarbon now available in sufficient quantity for biological studies. Interestingly, both of these reduced analogues 4 and 5 exhibited mutagenic activity in the Ames assay in the presence or absence of microsomal activation for strains TA98 and TA100. In these strains, DMBA was active only in the presence of S-9 fraction. In the plasmid-deficient strain TA1537, only tetrahydro analogue 5 exhibited mutagenic activity both in the absence and presence of S-9 fraction.

7,12-Dimethylbenz[*a*]anthracene (DMBA; 1) is one of the most potent known aromatic carcinogenic hydrocarbons.[3] Recent findings suggest that metabolism in the bay region (A ring in DMBA) plays a critical role in the mutagenicity and carcinogenicity of certain of these polycyclic hydrocarbons.[3-8] We have shown that substitution of F for H in the 2 and 5 positions, but not the 11 position, of DMBA reduces DNA binding in mammalian cultured cells relative to DMBA[9,10] and that such binding is in agreement with their relative oncogenic potential.[11] The recently synthesized dihydrodiol 2, which may serve as a metabolic precursor to epoxides such as 3, has been shown by Slaga et al.[12] to be the most carcinogenic hydrocarbon metabolite thus far tested.

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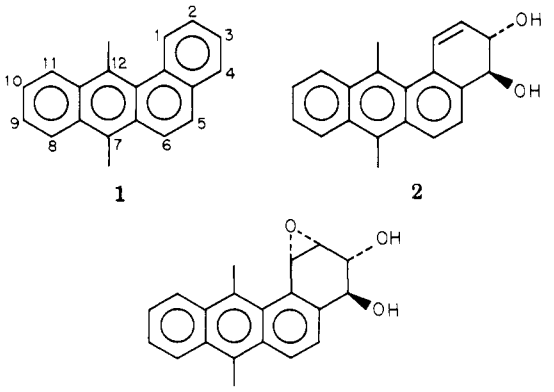
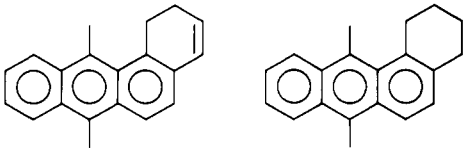


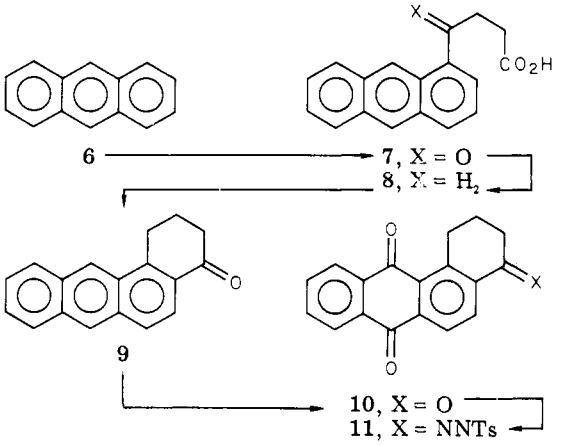
1 2 3

In this article, we describe the synthesis and mutagenic activity in the Ames assay for two A-ring reduced analogues of DMBA (1), namely, 1,2-H_2DMBA (4) and 1,2,3,4-H_4DMBA (5). We anticipated that their investigation could prove useful in studies relating to structural requirements for mutagenicity or carcinogenicity.



4 5

Chemistry. For the synthesis of 4, anthracene (6) served as starting material. Reaction of 6 with succinic



anhydride in CH_2Cl_2 in the presence of $AlCl_3$, followed by modified reaction workup, gave the known acid 7,[13] which upon Wolff–Kishner reduction was smoothly converted to 8 in 92% yield. In this case, Wolff–Kishner reduction was cleaner and provided higher yields than the reported Clemmenson reduction.[13] Cyclization of 8 to 9,[13] followed by CrO_3/AcOH oxidation to trione 10, was accomplished as reported.[14] Tosylhydrazone 11 was formed in quantative yield.

Transformation of 11 to dihydro compound 4 was carried out without isolation of intermediates 12 and 13. Conversion of aliphatic tosylhydrazones to olefins by reaction with alkyllithiums is well known.[15,16] Additionally, conversion of anthraquinones to their 9,10-dimethyldi-

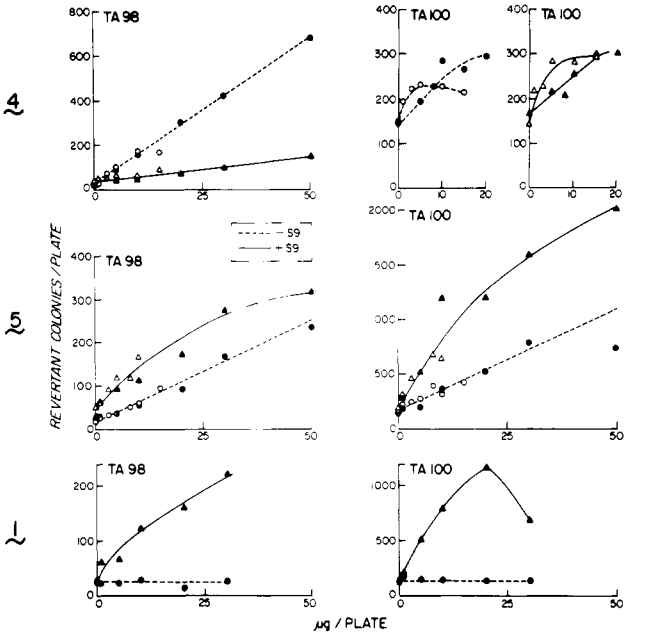


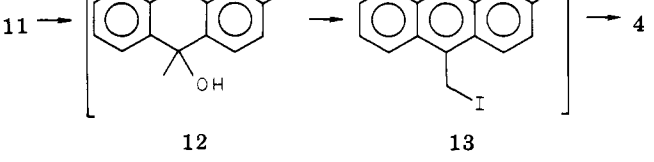
Figure 1. Mutagenicity for TA98 and TA100 of the dihydro (4) and tetrahydro (5) derivatives of 7,12-dimethylbenz[a]anthracene (1). Varying amounts of these compounds were incubated on the plate in the absence (dotted lines) and in the presence of activation mix containing 20 μL of liver microsomal S-9 fraction from polychlorobiphenyl (PCB) induced rats (solid lines). Open and solid symbols in the same graph indicate average values for two plates per point of repeat experiments.

Table I. Mutagenic Activity of DMBA and Derivatives

	net revertant colonies/10 μg of compd with each strain[a]					
	TA1537		TA98		TA100	
compd	−S9	+S9	−S9	+S9	−S9	+S9
dihydro derivative (4)	−	−	146	25	58[b]	69[b]
tetrahydro derivative (5)	42	7	47	78	173	270
DMBA (1)	−	15	−	58	−	489

[a] Values are taken from points on regression plots of linear dose-response from Figure 1 or as described in text. [b] Derived from experiment •, ▲ of Figure 1. Minus (−) indicates no mutagenic response. Compounds were not mutagenic for TA1535 and TA1538 at the concentration tested.

hydrodiols can be achieved by reaction with MeLi.[17] Thus, when 11 underwent reaction with 8 equiv of MeLi in THF



12 13

at room temperature overnight, intermediate 12 was expected following quenching with NH_4Cl. The crude product, after solvent removal, was dissolved in MeOH and treated with 47–49% HI. The expected iodomethyl analogue 13 was unstable to light and was immediately reduced with $NaBH_4$ following filtration. The desired H_2DMBA (4) was purified on neutral alumina and recrystallized from ethyl acetate, affording yellow crystals

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in 26% overall yield from tosylhydrazone 11. For metabolic studies, traces of DMBA (1) were removed from 4 by high-pressure LC on Partisil (Whatman) by elution with MeOH. Tetrahydro analogue 5 was synthesized by modifications of known procedures[18-20] from 1,2,3,4-tetrahydrobenz[a]anthraquinone (14; see Experimental Section).

Results and Discussion

Dose-dependent mutagensis for TA98 and TA100 for 4, 5, and 1 are plotted in Figure 1. These data are summarized in Table I where regression values from the linear portion of the curves are normalized to net revertant colonies per 10 μg of compound on the plate. As previously established,[21,22] mutagenesis of 1 in *S. typhimurium* is microsome mediated. Both of the reduced A-ring analogues reported here display appreciable dose-dependent activity in the presence of microsome activation, with 5 showing activity for TA98 which is at least as great as is seen for 1 assayed under the same conditions (Figure 1). Using strains TA1535, TA1537, and TA1538 in the presence and in the absence of microsomal activation, 1 and 5 were tested at up to 15 μg/plate, and 4 was tested at up to 10 μg/plate. None of these three compounds was mutagenic for either TA1535 or TA1538; values for dose-dependent mutagenesis obtained with TA1537 are presented in Table I.

Unlike 1, which displays only microsome-dependent mutagenic activity, 4 and 5 are potent mutagens for two or more strains in the absence of microsomal activation (Figure 1, Table I). Similar observations recently were reported by Malaveille et al.[23] for 2 using TA100. These investigators showed that for the low range of doses tested in the presence of a microsomal system, 2 showed about six times the activity of 1, an observation consistent with other evidence implicating 3 as the ultimate carcinogenic DMBA metabolite.[12,23-25] Notably, however, in the absence of microsome enzyme cofactors, 2 was mutagenic for TA100 at a level equal or greater than was observed for in vitro activated DMBA. In contrast, it would seem that 4 is metabolized by the S-9 fraction to materials less mutagenic for strain TA98 but not for strain TA100. Solutions of 4 were checked by high-pressure LC for autoxidation to 1 before and after assay, but no DMBA or other hydrocarbons were detected (see Experimental Section).

Curiously, the monomethyl analogue of 2, the 3,4-dihydrodiol of 7-methylbenz[a]anthracene, was reported to be nonmutagenic for TA98 when cofactors for microsomal monooxygenase were omitted.[23] Such observations and the strain-specific responses reported here support the use of multiple strains in *Salmonella* mutagenesis testing. As with DMBA, 4 and 5 are not mutagenic for the missense tester strain TA1535. Additionally, TA1538, which is relatively insensitive to DMBA mutagenesis,[21] was not mutated by any of the compounds in our study. Thus, mutagenesis of 4 and 5 is enhanced by the plasmid pKM101 which is present in TA98 and TA100. Nevertheless, it is clear that TA1537, which lacks the plasmid, is readily mutated by 5 in the absence of microsomal activation.

Not only is the relationship of these findings to mammalian metabolism in vivo unknown, but what relationship these observations bear on the bay-region mutagenesis theory is unclear. It may be that in bacteria these analogues (i.e., 4 and 5) undergo alternative routes of metabolite activation or deactivation. Availability of these two compounds should facilitate studies of the parallels of mutagenesis in procaryotic and eucaryotic systems and provide probes for the study of polycyclic hydrocarbon carcinogenesis. Other partially reduced polycyclic aromatic hydrocarbons are also of interest in this regard.[3,26] Studies with fluoro and methyl analogues of various polycyclic aromatic hydrocarbons[25-28] also reveal that alternative metabolic routes to bay-region metabolism are possible and, dependent upon the nature of enzyme inducers, different products can be formed in S-10 fractions.[29]

Experimental Section

Chemistry. All melting points are uncorrected. NMR spectra were obtained with a Varian A-56/60A spectrometer using $CDCl_3$ as solvent and tetramethylsilane as internal standard. All analyses (Galbraith Laboratories, Inc., Knoxville, Tenn.) obtained were within 0.2% of the theoretical values. Methyllithium was purchased from Ventron Co., Danvers, Mass. High-pressure liquid chromatography was carried out on a Laboratory Data Control (LDC) Gradient Master.

β-(1-Anthroyl)propionic Acid (7). A suspension of anthracene (71.4 g, 0.40 mol) and succinic anhydride (40 g, 0.40 mol) in CH_2Cl_2 (400 mL) was cooled in an ice bath, and $AlCl_3$ (55 g, 0.41 mol) was added in four portions over 20 min with vigorous mechanical stirring. The deep-red mixture was stirred for another 60 min at 0–5 °C and stored in the refrigerator overnight. EtOAc (400 mL), ice, and dilute HCl were added with stirring to decompose the complex. The mixture was digested on a steam bath for 2 h during which time the organic solvents evaporated. The mixture was cooled and decanted and the solid was washed thoroughly with H_2O. The solid was digested with dilute K_2CO_3 solution on a steam bath for 30 min and cooled, and the remaining anthracene (35 g) was collected by filtration. To the filtrate was added EtOAc (800 mL), followed by the dropwise addition of concentrated HCl with stirring. The layers were separated and the aqueous layer was washed with EtOAc. The combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The oily residue was triturated with a small amount of benzene, and the yellow precipitate was filtered and air-dried, affording 30.5 g (55%) of 7, mp 180–184 °C (lit.[13] mp 184–185 °C). Recrystallization from EtOAc raised the melting point to 184–185 °C, but the crude product (as judged by NMR) was sufficiently pure for conversion to 8.

γ-(1-Anthryl)butyric Acid (8). A mixture of 7 (27.8 g, 0.1 mol), KOH (50 g), 97% H_2NNH_2 (50 mL), and diethylene glycol (500 mL) was heated under reflux overnight. After the mixture was cooled, ice and dilute HCl solution were added and the acidified mixture was thoroughly extracted with EtOAc. The organic extracts were dried (Na_2SO_4) and evaporated to a small volume under reduced pressure. Petroleum ether (bp 35–60 °C) was added and the mixture chilled. Colorless crystals (24.3 g, 92%) of 8, mp 147–149 °C (lit.[13] mp 148–149 °C), were obtained fol-

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lowing filtration and drying in air.

1,2,3,4-Tetrahydrobenz[*a*]anthracen-4-one (9). Acid 8 was treated with PCl_5 in benzene and cyclized with $SnCl_4$ as previously described.[13] The product was extracted into benzene–EtOAc and purified by passing the solution through alumina (Fisher). The yellow solid was recrystallized from EtOAc [8.5 g (65%) from 13.2 g of 8], mp 195–197 °C (lit.[13] mp 196–197 °C).

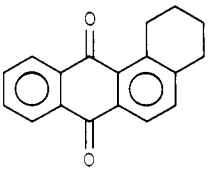
1,2,3,4-Tetrahydrobenz[*a*]anthracene-4,7,12-trione (10). To a suspension of 9 (2.46 g, 0.01 mol) in HOAc (35 mL) and H_2O (5 mL) was added dropwise, with stirring, a solution of CrO_3 (Baker; 5 g, 0.05 mol) in HOAc (25 mL) and H_2O (15 mL) over 30 min. The mixture was heated in an oil bath (70–75 °C) for 1 h and cooled. Dilution with H_2O and filtration of the solid, followed by recrystallization from EtOAc, yielded yellow crystals of 10 (1.71 g, 62%), mp 198–199 °C (lit.[14] mp 199 °C).

Reactions of 10 with Tosylhydrazine. To a mixture of 10 (0.55 g, 2.0 mmol) and tosylhydrazine (0.37 g, 2.0 mmol) in MeOH (10 mL) was added 4 drops of concentrated H_2SO_4. After heating at reflux with stirring for 1 h, the mixture was cooled and the yellow needles were filtered. Washing with cold MeOH followed by petroleum ether (bp 35–60 °C) afforded 11 (0.87 g, 98%): mp 239–241 °C dec; NMR ($CDCl_3$ plus a few drops of CF_3COOH) δ 2.4 (m, 2 H), 2.45 (s, 3 H), 2.7 (m, 2 H), 3.45 (m, 2 H), 7.4 (d, 2 H, J = 8 Hz), 7.7–8.5 (m, 8 H, aromatic). Anal. ($C_{25}H_{20}N_2O_4S$) C, H, N.

1,2-Dihydro-7,12-dimethylbenz[*a*]anthracene (4). To a solution of 11 (2.22 g, 5.0 mmol) in dry THF (freshly distilled from CaH_2; 200 mL), under N_2 with ice-cooling and stirring, was added a solution of MeLi in ether (1.6 M, 25.0 mL, 40.0 mmol) dropwise over 15 min. The yellow solution turned deep red upon addition of MeLi. The ice bath was removed and the mixture was stirred at room temperature under N_2 for 16 h. The reaction mixture was cooled and the excess MeLi decomposed by addition of ice. Saturated NH_4Cl solution (100 mL) was added with stirring and the layers were separated. The organic layer (protected from light) was dried (Na_2SO_4) and evaporated under reduced pressure. Subsequent operations were carried out in diffused light, and all reaction vessels were wrapped in aluminum foil.

The residue was dissolved in MeOH (10 mL), which was added dropwise to an ice-cold solution of 48% HI (25 mL) in MeOH (20 mL) with stirring. After 10 min, the yellow precipitate was quickly filtered, washed with H_2O (10 mL) and cold MeOH (10 mL), and rapidly transferred to a beaker containing $NaBH_4$ (2.0 g) and 95% EtOH (100 mL). The mixture was stirred at room temperature with exclusion of light for 2 h and then evaporated to a low volume (ca. 10 mL). H_2O (100 mL) was added and the mixture was stirred for 30 min. Extraction with benzene (2 × 40 mL), followed by passing the solution through neutral alumina (Fisher) and evaporation of the eluate, gave a yellow solid which on recrystallization from a small amount of EtOAc yielded 0.335 g (26%) of yellow crystals (4): mp 123–124 °C; NMR ($CDCl_3$) δ 2.0–2.45 (m, 2 H, H_2), 2.98 (s, 3 H, CH_3), 3.2–3.7 (m, 2 H, H_1), 6.1 (m, 1 H, H_3), 6.6 (m, 1 H, H_4), 7.1–7.6 (m, 3 H, aromatic), 8.0–8.3 (m, 3 H, aromatic); UV λ_{max} 300, 288, 273 nm. Anal. ($C_{20}H_{18}$) C, H. High-pressure LC analysis showed that the 7,12-dimethylbenz[*a*]anthracene was present as a trace impurity (0–5%). Pure dihydro compound for biological testing was obtained by high-pressure LC separation. Separation was carried out on a Partisil PXS 10/25 ODS-2 column (Whatman); methanol–water linear gradient (60–100%); linear; 20 min; DMBA eluted at 25 min and 1,2-H_2DMBA (4) eluted at 25.7 min.

1,2,3,4-Tetrahydrobenz[*a*]anthraquinone (14).[30] A mixture of 1,4-napthoquinone (3.16 g, 0.02 mol) and 1-vinylcyclohexene[18] (2.6 g, 0.024 mol) in MeOH (25 mL) was heated under reflux for 4 h. The mixture was concentrated to a small volume and cooled in ice. Intermediate 15 separated as white shiny needles (4.8 g,



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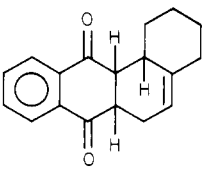

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90%), mp 102–103 °C (lit.[19] mp 102–103 °C). Air was bubbled through the stirred solution of the diketone 15 dissolved in dilute EtOH/KOH. The purple solution turned yellow within a few minutes. Dilution with H_2O led to the precipitation of quinone 14 in quantitative yield, mp 152–154 °C (lit.[20] 153–154 °C) without crystallization.

1,2,3,4-Tetrahydro-7,12-dimethylbenz[*a*]anthracene (5).[31] To a mixture of 14 (0.53 g, 2.0 mmol) and anhydrous Et_2O (10 mL) under N_2 was added dropwise a solution of MeLi in Et_2O (1.6 M, 6 mL) at room temperature with stirring. After stirring overnight under N_2, the fluorescent solution was treated with HI and $SnCl_2$ essentially as described in the literature[20] to give the title compound as yellow crystals (102 mg, 20%): mp 89–90 °C (lit.[20] mp 89–90 °C); NMR ($CDCl_3$) δ 1.40–2.10 (m, 4 H), 2.70–3.40 (m, 10 H), 6.98–7.55 (m, 3 H), 7.90–8.34 (m, 3 H). Anal. ($C_{20}H_{20}$) C, H.

Biology. Bacterial Mutagenesis. The *Salmonella*-microsome system has been described by Ames et al.,[32] who provided strains TA1535, TA1537, TA1538, TA98, and TA100. Strain TA1535 is an indicator of missense mutagenesis; strains TA1537, TA1538, and TA98 (which is TA1538 with the plasmid pKM101) are detectors of frame-shift mutagens. Similarly, TA100 is TA1535 containing pKM101. The presence of pKM101 increases the sensitivity of these strains to mutagenic compounds, and in TA100 its presence may alter specificity to include detection of frame shift as well as missense mutagens. Bacterial stocks were prepared weekly, and their characteristic mutagenesis[32,33] was verified for each fresh stock and again as part of each experiment. Preparation, testing, and storage (70 °C) of polychlorobiphenyl (PCB) induced rat liver microsomes (S-9 fraction)[32] was by modification of Ames' procedure, as described elsewhere.[33] Tests were conducted by standard soft agar platings from Me_2SO according to Ames et al.,[32] using triplicate plates for spontaneous mutagenesis and duplicate plates for each experimental point; five or more doses from 1 to ≥10 μg/plate were tested in the absence and presence of S-9 mix microsomal activation. A common pool of S-9 was used for all experiments, with the concentration fixed at 20 μL of S-9/plate. 2-Aminoanthracene and 1 served as positive controls of activation capacity in each experiment. Both 4 and 5 were tested two times with all five strains, except for 4 which was examined once using TA1535, TA1537, and TA1538. To be scored as mutagenic, a compound must have reverted the test strain in a dose-dependent fashion, producing numbers of colonies at least two times those obtained spontaneously. During these experiments, the average spontaneous revertant colonies/plate, minus and plus microsomal activation, were TA1535: 14, 11; TA1537: 8, 12; TA1538: 12, 28; TA98: 25, 33; TA100: 145, 167.

(30) This Experimental Section for 14 represents an improved synthesis over the published procedure (ref 19).

(31) This Experimental Section for 5 represents an improved synthesis over the published procedure (ref 20).

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