Unequivocal Synthesis of Euglenapterin

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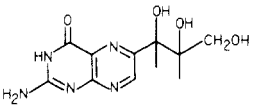
In a recent paper, Elstner and Heupel reported the isolation of three fluorescent compounds from *Euglena gracilis*, one of which stimulated ferredoxin-dependent oxygen reduction by isolated *Euglena* chloroplasts in the dark.[1] These compounds were tentatively identified as pteridine derivatives, although neither the compounds themselves nor their simple degradation products could be related to known members of this class (cf. neopterin (1)). In an elegant series of experiments, Pfleiderer et al.



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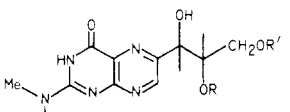
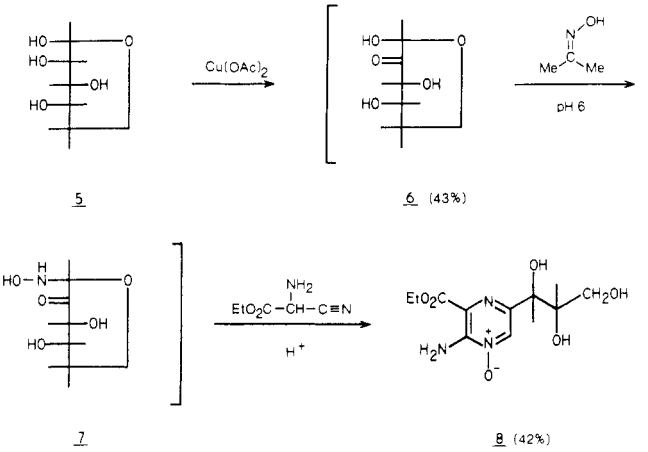


2 (R, R′ = H)
3 (R = H, R′ = PO_3H_2)
4 (R, R′ = Cyclophosphate)

have subsequently demonstrated that these compounds have structures **2**–**4**,[2] with the name euglenapterin (**2**) being given to the parent member of the series. These same authors also reported the first synthesis of **2** by conventional methods of pterin chemistry.[2,3] It is well-known, however, that such methods invariably lead to complex mixtures of products which frequently require laborious chromatographic separation. Since **2** is the first example of this novel class of naturally occurring pterins, wherein the 2-dimethylamino group might be of biological and physiological significance, we have investigated the synthesis of **2** by an unequivocal route.[4] This route allows for the synthesis of **2** in gram quantities, with no chromatographic separations, and opens the possibility for selective labeling at the key 8a angular carbon.

The key intermediate for our synthesis of **2** was the pyrazine *N*-oxide **8**, which was routinely prepared as follows (Scheme I).[4a,6] L-Xylose (**5**) was readily oxidized to L-xylosone (**6**) according to the procedure of Weidenhagen.[7] The mixture thus obtained was shown to contain 40–55% of **6** by conversion to iminoascorbic acid followed by titration with iodine.[4a,8] Without purification, osone **6** was

Scheme I



5 6 (43%) 7 8 (42%)

selectively converted to the aldoxime derivative **7** through an exchange reaction with acetone oxime in aqueous solution at pH 6. This method has previously been demonstrated to be an effective means for the preparation of aldoximes in the presence of other carbonyl functionalities.[4a] Condensation of **7** with ethyl α-aminocyanoacetate then gave 2-amino-3-(carboethoxy)-5-(L-*threo*-trihydroxypropyl)pyrazine 1-oxide (**8**) in 40–45% overall yield from **6**.[4,9]

The subsequent conversion of **8** to euglenapterin (**2**) presented unexpected difficulties (Scheme II). As indicated, **8** could be cleanly reduced to **9** with aqueous sodium dithionite. This latter material, however, failed to cyclize with 1,1-dimethylguanidine (**10**), giving open-chain analogue **11** as a stable, isolable species.[10] Interestingly, however, **8** itself reacted smoothly with **10** to give **13** as the only observable product. The loss of dimethylamine in this case is in contrast to results reported with *o*-amino nitriles, which give 2-(dimethylamino)pyrimidines under identical reaction conditions.[11,12] All attempts to circumvent this problem through the preparation of intermediate **14** were unsuccessful, presumably due to the low nucleophilicity of 1,1,3,3-tetramethylguanidine.[13]

Finally, **8** was successfully converted to **2** as indicated in Scheme III. Thus, **8** gave virtually quantitative yield of **15** upon dissolution in liquid NH_3, and this latter material was smoothly reduced to 2-amino-3-carbamoyl-5-(L-*threo*-trihydroxypropyl)pyrazine (**16**) with Raney Ni. Compound **16**, in turn, gave a 77% yield of euglenapterin (**2**) with tetramethylurea diethyl acetal[14] in DMF at ambient temperature. The material thus obtained was identical in all respects with an authentic sample of **2** kindly provided by Professor Pfleiderer.[15]

Experimental Section

Elemental analyses were carried out by the Baron Consulting Co. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. A Varian XL-200 spec-

(1) Elstner, E.; Heupel, A. *Arch. Biochem. Biophys.* **1976**, *173*, 614.
(2) Böhme, M.; Pfleiderer, W.; Elstner, E.; Richter, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 473.
(3) Viscontini, M.; Provenzale, R.; Ohlgart, S.; Mallevialle, J. *Helv. Chim. Acta* **1970**, *53*, 1202.
(4) (a) Taylor, E. C.; Jacobi, P. A. *J. Am. Chem. Soc.* **1976**, *98*, 2301. (b) Taylor, E. C.; Perlman, K. L.; Sword, I. P.; Sequin-Frey, M.; Jacobi, P. A. *Ibid.* **1973**, *95*, 6407.
(5) DeGraw, J.; Brown, V.; Uemura, I. *J. Labelled Compd. Radiopharm.* **1979**, *26*, 559.
(6) Satisfactory elemental analyes and spectral data were obtained for all new compounds reported. All yields refer to isolated and purified materials, except as noted.
(7) Weidenhagen, R. *Z. Wirtshaftsgruppe Zuckerind.* **1937**, *87*, 711. Yields for this reaction were found to vary with different samples of cupric acetate. Best results were obtained with material provided by the Fisher Scientific Co.
(8) (a) Salomon, L.; Burns, J.; King, C. *J. Am. Chem. Soc.* **1952**, *74*, 5161. (b) Hamilton, J.; Smith, F. *Ibid.* **1952**, *74*, 5162.
(9) This yield, which refers to the amount of **8** which precipitated directly from the reaction mixture, was somewhat variable. UV analysis, however, indicated that **8** was consistently formed in yields of >60%.
(10) The stability of such species has recently been commented on: Smith, R. L.; Cochran, D. W.; Gund, P.; Cragoe, E. J., Jr. *J. Am. Chem. Soc.* **1979**, *101*, 191.
(11) Taylor, E. C.; McKillop, A. "The Chemistry of Cyclic Enaminonitriles and *o*-Aminonitriles"; Interscience: New York, 1970.
(12) Weinstock, J.; Wiebelhaus, V. D. French Patent 1 335 354, Aug 16, 1963; *Chem. Abstr.* **1964**, *60*, 2975.
(13) Other reagents which failed to react with **8** include 2-phenyl-3,3-dimethylpseudourea, dimethylcyanamide, and tetramethylthiourea.
(14) Eilingsfeld, H.; Neubauer, G.; Seefelder, M.; Weidinger, H. *Chem. Ber.* **1964**, *97*, 1232.
(15) Fachbereich Chemie, Universität Konstanz, West Germany.

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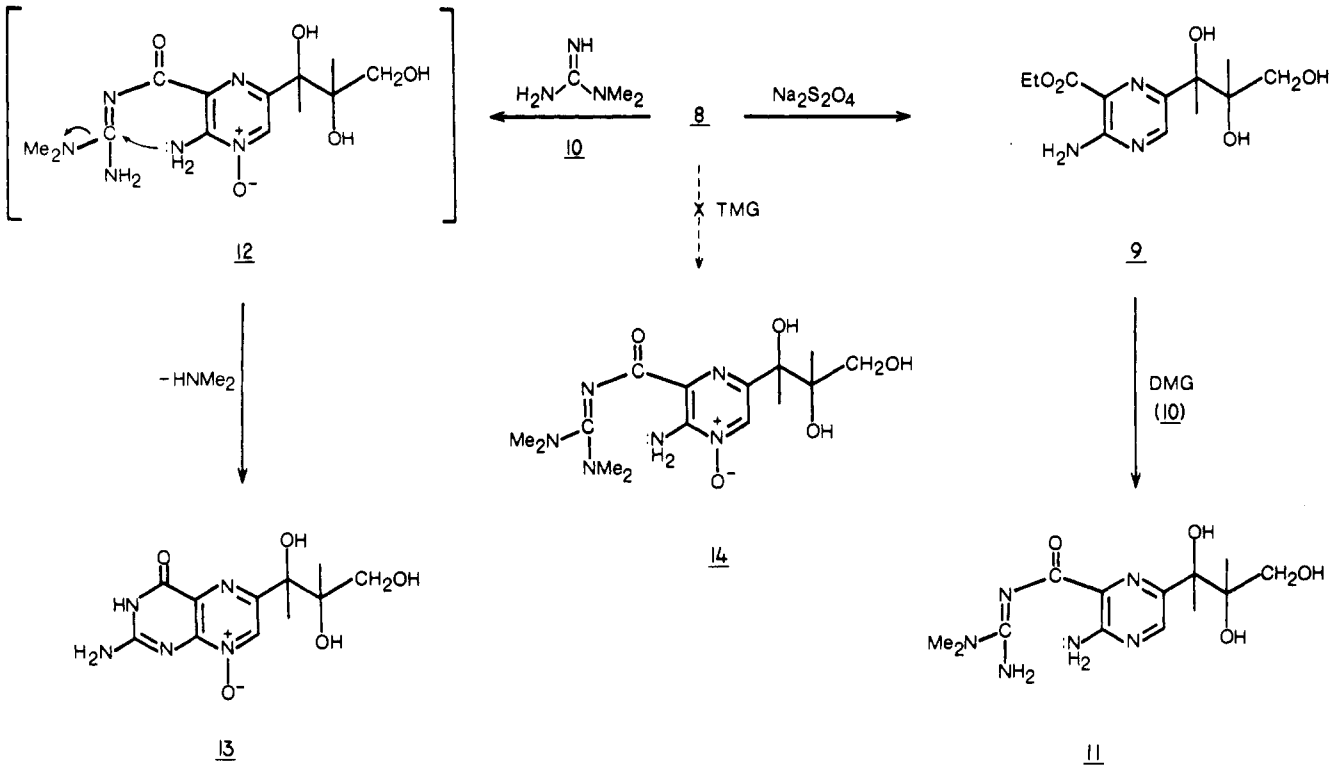
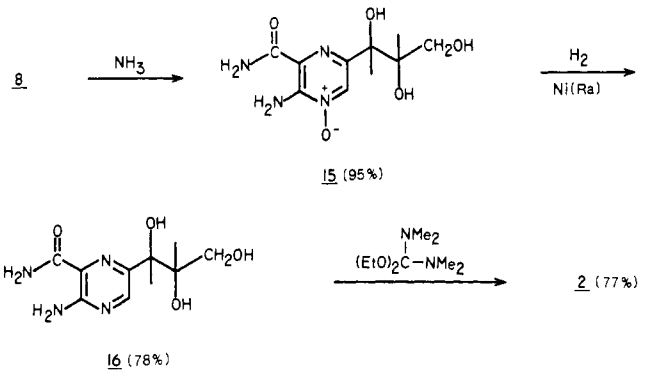
Scheme II



Scheme III



trometer, with solvents as indicated, was used for the NMR spectra, and UV spectra were recorded on a Carey 14 spectrophotometer.

L-Xylosone (6). A solution of 6.0 g (40.0 mmol) of L-xylose in 15 mL of water was diluted with 375 mL of methanol, and after the mixture was heated with stirring to a vigorous boil, 30.0 g (150.5 mmol) of cupric acetate hydrate was added in a single portion. The resulting suspension was boiled an additional 18 min before being cooled and filtered through Celite to remove cuprous oxide. The mother liquors were then filtered through a 2.5-cm column packed with 14 cm of Dowex 50-WX4 prepared in the usual manner (followed by thorough washing with methanol). The column was eluted with an additional 200 mL of methanol, and the clear colorless eluate was concentrated at 40 °C (10 torr) to a pale yellow syrup, from which residual acetic acid was removed by concentration from two 200-mL portions of water. In a blank experiment, the yield of **6** was determined as 43% on this scale.[4a] Somewhat higher yields (up to 55%) were obtained on a 8.0-mmole scale.

2-Amino-3-(carboethoxy)-5-(L-*threo*-trihydroxypropyl)-pyrazine 1-Oxide (8). The syrupy osone from 6.0 g (40.0 mmol) of L-xylose (**5**) was taken up in 25 mL of water, and the solution was adjusted to pH 7 by the dropwise addition of concentrated ammonium hydroxide. Acetone oxime (10.0 g, 137.0 mmol) was then added, and the resulting solution was stirred in an oil bath maintained at 50 °C. After a period of 14 h, the reaction mixture was clarified to a bright yellow with 200 mg of Darco G-60, diluted with an additional 75 mL of water, and extracted with three 50-mL portions of ether to remove excess acetone oxime. Concentration at 40 °C (10 torr) then gave a heavy orange gum which was dried under high vacuum for 2 h. The resulting material, consisting of crude L-xylosone aldoxime (**7**) was convered with 50 mL of absolute ethanol and warmed gently to effect partial solution. Ethyl α-aminocyanoacetate[4] (7.0 g, 23.5 mmol) was added, and the mixture was stirred at room temperature until solution (deep red) was complete. At this point stirring was stopped, and the reaction was allowed to stand at room temperature, with occasional scratching, until precipitation appeared complete (~36 h). The reaction mixture was then cooled for several hours, filtered, washed with ice-cold ethanol, and dried to give 1.96 g (42% overall based on **6**) of **8** as an off white crystalline solid.[9] The analytical sample crystallized from ethanol in the form of colorless elongated fibers: mp 180–82 °C; R_f 0.78 (2:1 *n*-PrOH/1% NH_3, cellulose); NMR (Me_2SO-d_6, D_2O) δ 8.33 (s, C_6 H, 1 H), 4.58 (d, J = 2.0 Hz, C_1' H, 1 H), 4.35 (q, J = 7.2 Hz, CH_2CH_3, 2 H), 3.57–3.29 (m, C_2' H, C_3' H, 3 H), 1.29 (t, J = 7.2 Hz, CH_2CH_3, 3 H). Anal. Calcd for $C_{10}H_{15}N_3O_6$: C, 43.95; H, 5.54; N, 15.38. Found: C, 43.78, H, 5.51; N, 15.35.

2-Amino-3-carbamoyl-5-(L-*threo*-trihydroxypropyl)-pyrazine 1-Oxide (15). A solution of 2.15 g (7.9 mmol) of ester **8** in 100 mL of liquid ammonia was stirred under reflux until all starting material had reacted (~3 h). The ammonia was then allowed to evaporate to dryness, and the residue was crystallized from 125 mL of ethanol to give 1.83 g (95%) of **15** as a slightly off-white crystalline solid. The analytical sample crystallized from ethanol in the form of colorless elongated fibers: mp 231–233 °C; R_f 0.49 (2:1 *n*-PrOH/1% NH_3, cellulose); NMR (Me_2SO-d_6, D_2O) δ 8.23 (s, C_6 H, 1 H), 4.56 (d, J = 2.8 Hz, C_1' H, 1 H), 3.71 (m, C_2' H, 1 H), 3.53–3.27 (ddd, J = 10.5, 6.0, 6.3 Hz, C_3' H, 2 H). Anal. Calcd for $C_8H_{12}N_4O_5$: C, 39.35; H, 4.95; N, 22.94. Found: C, 39.47; H, 4.88; N, 22.80.

2-Amino-3-carbamoyl-5-(L-*threo*-trihydroxypropyl)-pyrazine (16). A solution of 1.83 g (7.5 mmol) of amide **15** in 450 mL of 22% aqueous ethanol was treated with 1.8 g of W-2 Raney nickel,[16] and the resulting mixture was hydrogenated for 16 h at 45 psi of H_2. The reaction mixture was then filtered through Celite, washed with ethanol, and concentrated to a bright

(16) Mozingo, R. "Organic Syntheses"; Wiley: New York, 1955; Collect. Vol. III, p 181.

yellow powder. Crystallization of this material from ~50 mL of methanol gave 1.33 g (78%) of **16** in the form of short yellow needles: mp 176–77 °C; R_f 0.67 (2:1 *n*-PrOH/1% NH_3, cellulose); NMR (Me_2SO-d_6, D_2O) δ 8.15 (s, C_6 H, 1 H), 4.56 (d, J = 3.3 Hz, C_1' H, 1 H), 3.79–3.63 (m, C_2' H, 1 H), 3.55–3.17 (ddd, J = 10.8, 5.6, 6.4 Hz, C_3' H, 2 H). Anal. Calcd for $C_8H_{12}N_4O_4$: C, 42.10; H, 5.30; N, 24.55. Found: C, 42.25; H, 5.15; N, 23.79.

Euglenapterin (2). A suspension of 1.22 g (5.35 mmol) of amide **16** in 5 mL of dry DMF was treated with 10.19 g (53.5 mmol, 10 equiv) of tetramethylurea diethyl acetal.[14] After the suspension was stirred for 15 min, solution was complete, and after a total of 4 h the dark yellow-brown solution was concentrated to dryness under high vacuum. The resulting brown oil was concentrated three times from ethanol and three times from water (100 mL each) to remove excess acetal. The residue was then dissolved in 50 mL of water, treated with Darco G-60, filtered, and adjusted to pH 3.5 with 2 N HCl. After the mixture cooled overnight, filtration afforded 0.67 g of **2** as a yellow microcrystalline solid. Concentration to half volume gave an additional 0.48 g of **2** of equal purity (total yield 1.15 g, 77%). The material thus obtained had identical chromatographic and spectroscopic properties with the naturally occurring substance:[15] R_f 0.46 (2:1 *n*-PrOH/NH_3, cellulose); NMR (Me_2SO-d_6, D_2O) δ 8.72 (s, C_7 H, 1 H), 4.74 (d, J = 3.3 Hz, C_1' H, 1 H), 3.78–3.66 (m, C_2' H, 1 H), 3.58–3.32 (ddd, J = 10.8, 6.0, 6.0 Hz, C_3' H, 2 H), 3.07 (s, $N(CH_3)_2$, 6 H); UV λ_{max} (0.1 N NaOH) 381 nm, 272, λ_{max} (0.1 N HCl) 327 nm, 289 (w), 247. Synthetic **2** decomposed over a broad range beginning at ~206 °C.

Acknowledgment. Financial support of this work by Wesleyan University is gratefully acknowledged. The Varian XL-200 spectrometer used in this work was financed in part by the National Science Foundation (Grant No. CHE-7908593), the Dreyfus Foundation, and Wesleyan University.

Registry No. **2**, 73789-39-6; **6**, 26188-06-7; **8**, 79233-52-6; **15**, 79233-53-7; **16**, 79233-54-8; L-xylose, 609-06-3.

Structure and Spectroscopic Properties of So-Called α-Anthrapinacolin

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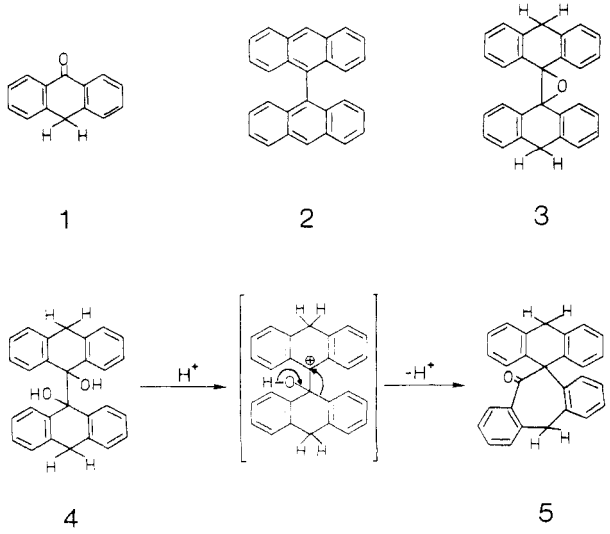
The reductive dimerization of anthrone (**1**) by zinc in the presence of mineral acid has been described to give 9,9′-dianthryl (**2**) and, in about 50% yield, the so-called α-anthrapinacolin (**3**).[1] Concerning the formation of an



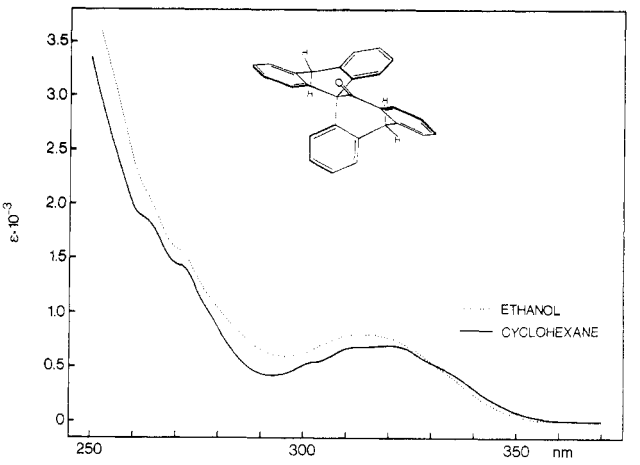


Figure 1. Electronic absorption spectra of anthrapinacolin **5** in cyclohexane (solid line) and in ethanol (dotted line).

anthrapinacolin from anthrone, a reviewer[2] had deemed it conceivable that the intermediate anthrapinacol (**4**) had undergone cationic rearrangement to give the seven-membered ketone **5**, but "this speculation" was rejected[3] as being devoid of any experimental basis. Indeed, a later investigation of anthrapinacolin also failed to provide chemical evidence for the presence of a carbonyl group.[4] Subsequently, anthrapinacolin **3** was described to be directly accessible from anthrapinacol (**4**) by treatment with thionyl chloride,[5] and as recently as 1973 the IR, UV, and ^1H NMR spectral data of anthrapinacolin obtained by this latter method were reported and believed to be in agreement with structure **3**.[6]

An interest in the electron spectral properties of the 9,10-dihydroanthracene chromophore[7] prompted us to investigate the structure of anthrapinacolin. We have confirmed that anthrapinacolin obtained by treatment of anthrone with zinc in the presence of acid indeed is identical with the product of dehydration of anthrapinacol by thionyl chloride. We also found anthrapinacolin to be nonfluorescent, as had been noted most recently.[6] All other spectroscopic data for anthrapinacolin, though in perfect agreement with the literature,[6] are from our point of view obviously at variance with the accepted epoxide structure **3**. Thus, the IR spectrum of anthrapinacolin (in KBr) exhibits a strong band at 1680 cm^{-1} which is indicative of an α,β-unsaturated carbonyl group. The UV spectrum of anthrapinacolin in cyclohexane solution exhibits a weakly structured absorption maximum around 315 nm (ε 690) which we attribute to an enhanced n–π* transition typical of a β,γ-unsaturated carbonyl chromophore.[8] In agreement with this assignment is the solvent dependence of the absorption spectrum (see Figure 1). In ethanol solution, the absorption maximum characteristic of the carbonyl n–π* transition is shifted hypsochromically as the onset of the π–π* transition is shifted toward lower energy. The presence of a carbonyl group in anthrapinacolin is also supported by its ^{13}C NMR spectrum (see Experimental Section).

Convincing evidence for structure **5**, rather than **3**, was obtained by ^1H NMR spectroscopy. The integration of the

(1) Barnett, E. d. B.; Mathews, M. A. *J. Chem. Soc.* **1923**, *123*, 380.
(2) Robinson, R. *Annu. Rep. Chem. Soc. (London)* **1923**, *20*, 121–122.
(3) Cook, J. W. *J. Chem. Soc.* **1928**, 58.
(4) Bergmann, E.; Schuchardt, W. *Justus Liebigs Ann. Chem.* **1931**, *487*, 225.
(5) Bell, F.; Waring, D. H. *J. Chem. Soc.* **1949**, 1579.
(6) Carlson, S. A.; Hercules, D. M. *Anal. Chem.* **1973**, *45*, 1794.
(7) Becker, H.-D.; Andersson, K.; Sandros, K. *J. Org. Chem.* **1980**, *45*, 4549.
(8) Houk, K. N. *Chem. Rev.* **1976**, *76*, 1.

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