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Bioorganic & Medicinal Chemistry Letters 15 (2005) 2331–2334
Bioorganic & Medicinal Chemistry Letters

N-Hydroxy sulfonimidamides as new nitroxyl (HNO) donors

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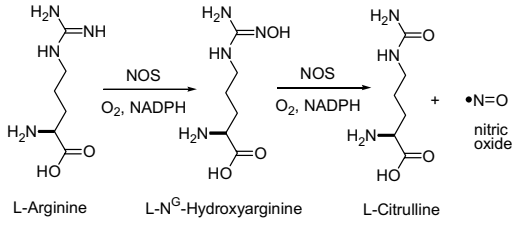
Received 26 January 2005; revised 24 February 2005; accepted 28 February 2005
Available online 25 March 2005

Abstract—Chlorination and condensation of simple sulfinamides with *O*-benzyl and *O*-*tert*-butyl dimethyl siloxy hydroxylamine gives *O*-protected *N*-hydroxy sulfonimidamides. Deprotection of these compounds produces the corresponding sulfinamide and nitrous oxide, which provides evidence for the intermediacy of nitroxyl (HNO) and identifies these compounds as new potential HNO donors.

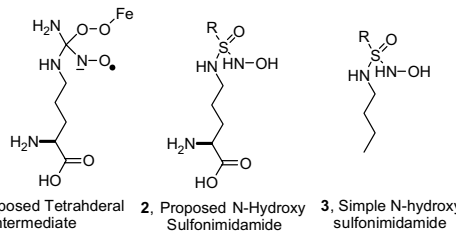
1. Introduction

The nitric oxide synthases (NOS) catalyze the oxidation of the terminal guanidino group of L-arginine to nitric oxide (NO), a neutral molecule that plays important roles in blood pressure control, neurotransmission, and the immune response (Scheme 1).[1] The first step in the conversion of L-arginine to NO, *N*-hydroxylation of L-arginine to N^G-L-hydroxyarginine, requires two NADPH-derived electrons to activate molecular oxygen and finds precedence in the cytochrome P450 catalyzed *N*-hydroxylation of amidines.[2] This conversion follows a classic P450 type mechanism including formation of peroxo and perferryl iron heme complexes, hydrogen atom abstraction, and oxygen rebound.[1]

The second step, oxidation of N^G-L-hydroxyarginine to NO and L-citrulline is not as well understood and recent work reveals a unique redox role for the H_4B co-factor.[3] A number of potential mechanisms featuring activated iron heme-based oxidants and both oxygen and nitrogen centered radicals exist and have been summarized.[4] These mechanisms generally include a peroxy-iron species that reacts with either N^G-hydroxy-L-arginine or an N^G-hydroxy-L-arginine-derived radical to give a tetrahedral intermediate (**1**) that decomposes to L-citrulline and NO.[4] Given the presence of tetrahedral intermediates in these proposals, tetrahedral transition state analogs of N^G-L-hydroxyarginine, such as the L-arginine derived *N*-hydroxy sulfonimidamide (**2**) may be useful mechanistic probes for the NOS catalyzed conversion of N^G-L-hydroxyarginine to NO and potential NOS inhibitors. Also, the recent identification of nonamino acid derived *N*-hydroxyguanidines as competent NO producing substrates of NOS indicate that simple *N*-hydroxy sulfonimidamides, such as **3**, may also act as transition state analogs.[5] We report synthetic approaches toward *N*-hydroxy sulfonimidamides and their unexpected rapid decomposition to the corresponding sulfinamide and nitroxyl (HNO), the one-electron reduced form of NO. These results identify *N*-hydroxy sulfonimidamides as a



Scheme 1. NOS catalyzed oxidation of L-arginine to NO.



Keywords: Nitroxyl; Nitric oxide; Piloty's acid; Nitric oxide synthase.
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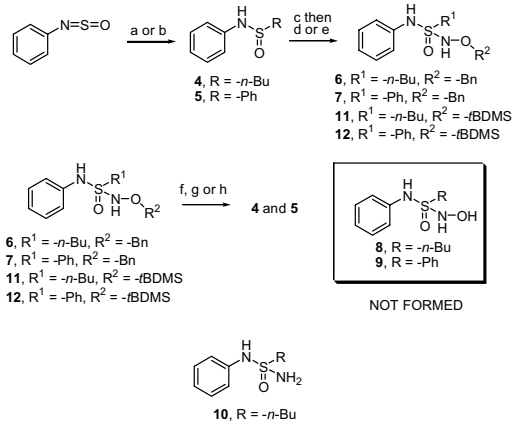
doi:10.1016/j.bmcl.2005.02.082



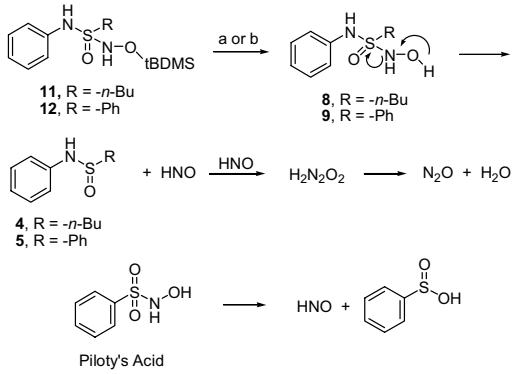
Scheme 2. Reagents: (a) *n*-BuLi; (b) PhMgBr; (c) *t*-BuOCl; (d) $BnONH_2$; (e) *t*-$BDMSONH_2$; (f) Pd/C, H_2; (g) TBAF; (h) CH_3CO_2H–H_2O–THF.

new group of potential donors of HNO, which has received considerable recent interest given its different biological profile from NO. The chemistry and biology of HNO has recently been extensively reviewed.[6]

2. Results and discussion

Scheme 2 depicts the initial attempt at the preparation of model *N*-hydroxy sulfonimidamides. Treatment of *N*-thionylaniline with either *n*-butyl lithium or phenyl magnesium bromide yields the sulfinamides (**4** and **5**) in 82% and 73% yield, respectively (Scheme 2).[7,8] Exposure of **4** and **5** to *t*-butyl hypochlorite followed by condensation with *O*-benzyl hydroxylamine gives the corresponding benzyl protected *N*-hydroxy sulfonimidamides (**6** and **7**) in 59% and 67% yield, respectively (Scheme 2).[9,10] Catalytic hydrogenation (10% Pd/C catalyst, room temperature, 1 atm of H_2, CH_3OH) of **6** and **7** does not form the expected *N*-hydroxy sulfonimidamides (**8** and **9**) but surprisingly produces the corresponding sulfinamides (**4** and **5**) as judged by TLC and ^1H NMR spectroscopy as compared to authentic samples (Scheme 2). The preparation of **10** by treatment of **4** with *t*-butyl hypochlorite followed by ammonia and ^1H NMR and TLC characterization eliminates **10**, which could form by N–O bond reduction, as the reaction product.

Scheme 2 also summarizes the alternative use of the *t*-butyl dimethyl silyloxy (*t*-BDMS) protecting group in the preparation of *N*-hydroxy sulfonimidamides. Exposure of **4** and **5** to *t*-butyl hypochlorite followed by condensation with O–*t*-BDMS hydroxylamine yields the O–*t*-BDMS protected *N*-hydroxy sulfonimidamides (**11** and **12**) in 48% and 41% yield, respectively (Scheme 2). Treatment of **11** and **12** with tetra-*n*-butyl ammonium fluoride (TBAF) again gives the sulfinamides **4** and **5** with no indication of the *N*-hydroxy sulfonimidamides (**8** and **9**, Scheme 2). Exposure of **11** and **12** to acidic conditions (3:1:1, CH_3CO_2H–H_2O–THF, room temperature) only yields **4** and **5** with no evidence of **8** and **9** (Scheme 2). Finally, treatment of the sulfinamide **4** with *t*-butyl hypochlorite followed by the direct addition of hydroxylamine fails to give the desired *N*-hydroxy sulfonimidamide **8**.



Scheme 3. Reagents: (a) TBAF; (b) CH_3CO_2H–H_2O–THF.

Gas chromatographic analysis of the headspace of either the TBAF or acidic deprotection of **11** and **12** reveals the formation of nitrous oxide (N_2O, 75% and 65%, respectively, after 2 h, Scheme 3).[11] The identification of N_2O during these reactions provides strong evidence for the intermediacy of nitroxyl (HNO), which rapidly dimerizes and dehydrates to give N_2O.[12] The addition of an aqueous solution of TBAF to a solution of **11** and **12** in 1:1 CH_3OH–H_2O also produces N_2O in 51% and 55% yield, respectively, demonstrating the aqueous decomposition of **11** and **12** to HNO. Addition of glutathione (2 equiv) to these reaction mixtures completely suppresses N_2O formation providing further evidence of the intermediacy of HNO. These results suggest that removal of the protecting group of **11** and **12** initially forms the *N*-hydroxy sulfonimidamides **8** and **9** that immediately decompose to HNO and the sulfinamides **4** and **5** (Scheme 3). In retrospect, the decomposition of **8** and **9** to HNO may have been anticipated given the structural similarity of **8** and **9** to Piloty's acid, a compound known to decompose to HNO and benzene sulfinic acid under basic conditions (Scheme 3).[13]

These synthetic studies show the general instability of *N*-hydroxy sulfonimidamides, which severely limits their use as transition state analogs for NOS. However, these experiments reveal that this unique functional group acts as an HNO donor. Thus, *O*-protected *N*-hydroxy sulfonimidamides (such as **6**, **7**, **11**, and **12**) represent prodrugs capable of HNO release upon deprotection (dealkylation) similar to *N*,*O*-diacylated or alkylated *N*-hydroxyarylsulfonamides previously reported by Nagasawa and co-workers.[14] While Angeli's salt (sodium trioxodinitrate, $Na_2N_2O_3$) and Piloty's acid remain the most widely used HNO donors, the intense biological, chemical, and theoretical interest in HNO highlights the need for new HNO donors as biochemical and pharmacological probes and possible therapeutic agents.[6,15] For example, HNO and NO donors demonstrate discrete effects in both normal and failing heart models with HNO donors specifically increasing levels of calcitonin gene related peptide and NO donors increasing cyclic guanylate monophosphate levels.[16] These results have led to the suggestion of HNO donors as a new and unique

treatment for heart failure.[15] These results describe a new functional group (*N*-hydroxy sulfonimamides) as an alternative HNO donor that may be useful in distinguishing the effects of NO from HNO in some systems.

3. *N*-Phenylbutane sulfinamide (4)

N-Butyllithium (5.74 mL of a 2.5 M solution in hexanes, 1.44×10^{-2} mol was added to a stirred solution of *N*-thionylaniline (0.81 mL, 7.2×10^{-3} mol) in THF (5 mL) at −78 °C (acetone–dry ice bath). The reaction mixture was monitored by TLC and after 30 min. was carefully worked up by the addition of water, diluted with ether and extracted with 1 M HCl, $NaHCO_3$ (satd) and water. The organic layers were combined, dried ($MgSO_4$), concentrated, and purified by flash chromatography (silica gel, 50:50 pentane–ether, R_f 0.23) to give 1.20 g (84.5%) of **4** as a white powder: mp 73–74 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32–7.26 (m, 2H, ArH), 7.08–7.04 (m, 3H, ArH), 5.85 (br s, 1H, NH), 2.97–2.91 (m, 2H, α-CH_2), 1.81–1.71 (p, 2H, J = 7.59 Hz, β-CH_2), 1.56–1.46 (m, 2H, χ-CH_2), 1.00–0.95 (t, 3H, J = 7.31 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 141.0, 129.5, 123.4, 118.8, 56.0, 25.0, 21.8, 13.7; LRMS (ESI) *m*/*z* 198 (MH^+).

4. *N*-Phenylbenzenesulfinamide (5)

Phenylmagnesium bromide (3.6 mL of a 3 M solution in THF, 1.08×10^{-2} mol) was added to a stirred solution of *N*-thionylaniline (0.81 mL, 7.18×10^{-3} mol) in THF (5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then at room temperature for 2 h, with monitoring by TLC until completion. The reaction mixture was worked up by the addition of water, dilution with ether, and extraction with 1 M HCl, $NaHCO_3$ (satd) and water. The organic layers were combined, dried ($MgSO_4$), concentrated and he resultant oil was purified by flash chromatography (silica gel, 50:50 pentane–ether R_f 0.31), to yield 1.13 g (72.8%) of **5** as a white solid: mp 108–111 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83–7.81 (m, 2H, ArH), 7.56–7.55 (m, 3H, ArH), 7.32–7.29 (m, 2H, ArH), 7.12–7.07 (m, 3H, ArH), 6.06 (br s, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 131.8, 129.9, 129.5, 125.8, 124.1, 119.4. LRMS (ESI) *m*/*z* 239 (M+Na^+).

5. Protected *N*-hydroxy sulfonimidamides (6, 7, 11, 12)

t-Butyl hypochlorite (1.2 equiv) in CCl_4 (1 mL) was added dropwise to a solution of the sulfinamide (1 equiv) in CCl_4 (2 mL) and stirred at 0 °C in an aluminum foil-covered flask. After stirring for 1.5 h, the mixture was concentrated and the resultant residue was taken up in CCl_4 (2 mL) at room temperature. A solution of benzyl hydroxylamine or *t*-butyldimethylsilyl hydroxylamine (2.4 equiv) in CCl_4 was added to this solution dropwise. After stirring overnight, the reaction mixture was diluted with ether and extracted with 1 M HCl, satd $NaHCO_3$, and water. The organic layers were combined, dried ($MgSO_4$), concentrated, and purified by flash chromatography to give the protected *N*-hydroxysulfonimidamides (**6**, **7**, **11**, **12**) in 41–67% yield.

Compound **6**: R_f 0.69 (50:50 pentane–ether); mp 68–69 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51–7.20 (m, 9H, ArH), 7.02–7.00 (t, 1H, J = 7.20 Hz, ArH), 6.86 (br s, 1H, NH), 4.87–4.80 (m, 2H, CH_2OPh), 3.37–3.32 (m, 2H, α-CH_2), 1.91–1.86 (m, 2H, β-CH_2), 1.52–1.47 (sext., 2H, J = 7.51 Hz, χ-CH_2), 0.99–0.96 (t, 3H, J = 7.40 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 145.5, 129.7, 129.3, 129.0, 128.8, 124.1, 122.8, 79.2, 51.4, 23.3, 21.8, 13.9; LRMS (ESI) *m*/*z* 319 (MH^+).

Compound **7**: 0.75 (50:50 pentane–ether); mp 100–103 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17–8.15 (m, 2H, ArH), 7.64–7.53 (m, 3H, ArH), 7.35–7.29 (m, 7H, ArH), 7.25–7.20 (m, 2H, ArH), 7.08–7.05 (m, 1H, ArH), 6.91 (br s, 1H, NH), 4.79–4.78 (m, 2H, CH_2OPh), ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 143.4, 137.6, 136.0, 133.6, 129.5, 129.3, 128.9, 128.8, 128.8, 124.4, 123.1, 79.0; LRMS (ESI) *m*/*z* 339 (MH^+).

Compound **11**: R_f 0.53 (50:50 pentane–ether); mp 78–81 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23–7.20 (t, 2H, J = 7.75 Hz, ArH), 7.15–7.13 (d, 2H, J = 7.80 Hz, ArH), 6.96 (t, 1H, J = 7.79 Hz), 6.43 (br s, 1H, NH), 3.36–3.34 (m, 2H, α-CH_2), 1.94–1.91 (p, 2H, J = 7.64 Hz, β-CH_2), 1.54–1.53 (m, 2H, χ-CH_2), 1.02–0.99 (t, 3H, J = 7.57 Hz, CH_3), 0.93 (s, 9H, $SiC(CH_3)_3$), 0.17 (s, 3H, $SiCH_3$), 0.08 (s, 3H, $SiCH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 143.5, 129.0, 123.8, 122.3, 50.0, 26.1, 25.2, 21.7, 18.2, 13.7; LRMS (ESI) *m*/*z* 343 (MH^+).

Compound **12**: R_f 0.75 (50:50 pentane–ether); mp 91–93 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17–8.15 (m, 2H, ArH), 7.67–7.65 (m, 3H, ArH), 7.30–7.22 (m, 4H, ArH), 7.06–7.00 (m, 1H, ArH), 6.56 (br s, 1H, NH), 0.89 (s, 9H, $SiC(CH_3)_3$), 0.07 (s, 3H, $SiCH_3$), 0.01 (s, 3H, $SiCH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 143.0, 136.7, 133.4, 129.1, 129.1, 128.9, 124.1, 122.6, 26.0, 18.0; LRMS (ESI) *m*/*z* 363 (MH^+).

6. *N*-Phenylbutane sulfonimamide (10)

Prepared in a similar fashion to **6**, **7**, **11**, and **12** with ammonia being added to give **10**: R_f 0.75 (ether); mp 125–128 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33–7.25 (m, 2H, Ar), 7.18–7.15 (m, 2H, Ar), 7.07–7.02 (t, 1H, J = 7.25 Hz, Ar), 4.42 (br s, 1H, NH), 3.28–3.23 (m, 2H, α-CH_2), 1.97–1.88 (m, 2H, β-CH_2), 1.50–1.42 (m, 2H, J = 7.36 Hz, χ-CH_2), 0.99–0.94 (t, 3H, J = 7.33 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 129.6, 123.3, 122.9, 56.0, 26.3, 21.7, 14.0; LRMS (ESI) *m*/*z* 213 (MH^+), 235 (M+Na^+).

7. Attempted deprotection of 11 and nitrous oxide detection

A solution of tetra-*n*-butyl ammonium fluoride (4.38 mL, 1 M, 4.37×10^{-3} mol) in THF was added

dropwise to a solution of **11** (500 mg, 2.18×10^{-3} mol) in THF (10 mL) at room temperature. After stirring for 15 min, the reaction was judged complete by TLC. The reaction mixture was diluted with ether and extracted with water, and the ether layer was subsequently dried ($MgSO_4$) and concentrated to give a residue that was purified by flash chromatography (silica gel; 50:50 pentane–ether) to yield 255 mg (52%) of **4** as judged by TLC and NMR spectroscopy. An aliquot (250 μL) of the reaction headspace from a sealed reaction flask was injected onto a 6890 Hewlett–Packard gas chromatograph equipped with a thermal conductivity detector and a 6 ft X 1/8 in. Porapak Q column at an operating oven temperature of 50 °C (injector and detector 150 °C) with a flow rate of 16.67 mL/min (He, carrier gas). The retention time of nitrous oxide was 2.61 min and identical to a known sample. Yields were determined using a standard curve generated from known amounts of nitrous oxide.

Acknowledgements

This work was supported by grants (0140020N, Established Investigator Award) from the American Heart Association and the National Institutes of Health (HL62198).

References and notes

1. (a) Kerwin, J. F., Jr.; Lancaster, J. R., Jr.; Feldman, P. F. *J. Med. Chem.* **1995**, *38*, 4343; (b) Stuehr, D. J.; Santolini, J.; Wang, Z. Q.; Wei, C. C.; Adak, S. *J. Biol. Chem.* **2004**, *279*, 36167; (c) Stuehr, D. J. *J. Nutr.* **2004**, *134*, 2748S.
2. Clement, B.; Immel, M.; Pfunder, H.; Schmitt, S.; Zimmerman, M. In *New Aspects of the Microsomal N-Hydroxylation of Benzamidines in N-Oxidations of Drugs: Biochemistry, Pharmacology, Toxicology*; Chapman and Hall: London, 1991; pp 185–205.
3. (a) Wei, C. C.; Wang, Z. H.; Hemann, C.; Hille, R.; Stuehr, D. J. *J. Biol. Chem.* **2003**, *278*, 46668; (b) Hurshman, A. R.; Krebs, C.; Edmondson, D. E.; Marletta, M. A. *Biochemistry* **2003**, *42*, 13287.
4. (a) Rosen, G. M.; Tsai, P.; Pou, S. *Chem. Rev.* **2002**, *102*, 1191; (b) Huang, H.; Hah, J. M.; Silverman, R. B. *J. Am. Chem. Soc.* **2001**, *123*, 2674.
5. (a) Renodon-Corniere, A.; Boucher, J. L.; Dijols, S.; Stuehr, D. J.; Mansuy, D. *Biochemistry* **1999**, *38*, 4663; (b) Dijols, S.; Perollier, C.; Lefevre-Groboillot, D.; Pethe, S.; Attias, R.; Boucher, J. L.; Stuehr, D. J.; Mansuy, D. *J. Med. Chem.* **2001**, *44*, 3199; (c) Xian, M.; Fujiwara, N.; Wen, Z.; Cai, T. W.; Kazuma, S.; Janczuk, A. J.; Tang, X. P.; Telyatnikov, V. V.; Zhang, Y. X.; Chen, X. C.; Miyamoto, Y.; Taniguchi, N.; Wang, P. G. *Bioorg. Med. Chem.* **2002**, *10*, 3049; (d) Jia, Q.; Cai, T. W.; Huang, M. C.; Li, H. Y.; Xian, M.; Poulos, T. L.; Wang, P. G. *J. Med. Chem.* **2003**, *46*, 2271.
6. Miranda, K. M. *Coord. Chem. Rev.* **2005**, *249*, 433.
7. Kresze, G.; Moshke, H. *Angew. Chem., Int. Ed. Engl.* **1962**, *1*, 89.
8. Gilman, H.; Morris, H. *J. Am. Chem. Soc.* **1926**, *48*, 2399.
9. Mintz, M. J.; Walling, C. *Org. Synth. Coll. Vol. V.* **1973**, 184.
10. Johnson, C.; Wambsgans, A. *J. Org. Chem.* **1979**, *44*, 2278.
11. Huang, J.; Sommers, E.; Kim-Shapiro, D. B.; King, S. B. *J. Am. Chem. Soc.* **2002**, *124*, 3473.
12. (a) Smith, P. A. S.; Hein, G. E. *J. Am. Chem. Soc.* **1960**, *82*, 5731; (b) Kohout, F. C.; Lampe, F. W. *J. Am. Chem. Soc.* **1965**, *87*, 5795.
13. Bonner, F. T.; Ko, Y. *Inorg. Chem.* **1992**, *31*, 2514.
14. (a) Fukuto, J. M.; Hszieh, R.; Gulati, P.; Chiang, K. T.; Nagasawa, H. T. N. *Biochem. Biophys. Res. Commun.* **1992**, *187*, 1367; (b) Lee, M. J. C.; Nagasawa, H. T.; Elberling, J. A.; Demaster, E. G. *J. Med. Chem.* **1992**, *35*, 3648–3652; (c) Nagasawa, H. T.; Kawle, S. P.; Elberling, J. A.; Demaster, E. G.; Fukuto, J. M. *J. Med. Chem.* **1995**, *38*, 1865.
15. Feelisch, M. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4978.
16. (a) Paolocci, N.; Katori, T.; Champion, H.; St. John, M. E.; Miranda, K. M.; Fukuto, J. M.; Wink, D. A.; Kass, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5537; (b) Miranda, K. M.; Paolocci, N.; Katori, T.; Thomas, D. D.; Ford, E.; Bartberger, M. D.; Espey, M. G.; Kass, D. A.; Feelisch, M.; Fukuto, J. M.; Wink, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9196.