Journal of the Chinese Chemical Society, **1998**, *45*, 549-553

A General Procedure for Synthesis of N^G-Alkyl, and N^G-Aryl-L-Arginines as Potential Nitric Oxide Synthase Inhibitors

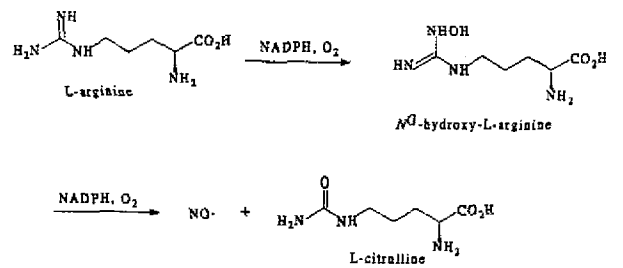
Bor-cherng Chen (陳柏成), Shi Shiu (徐 石) and Ding-yah Yang* (楊定亞)
Department of Chemistry, Tunghai Christian University, Taichung, Taiwan 40704, R.O.C.

A general procedure for the synthesis of N^G-alkyl, and N^G-aryl-L-arginines with relatively high overall yield is reported. The key step involved the coupling of protected L-ornithine **4** with isothiourea **7** to give the fully protected N^G-aryl-L-arginine derivative **8**. Subsequent deprotection of **8** in acidic condition provided the final target compound **9** with an overall yield of more than 80%.

INTRODUCTION

Nitric oxide has been identified as a multifunctional mediator with ubiquitous presence in most cells of the human body.[1] It has been described as an endothelium-derived relaxing factor (EDRF),[2] a neutotransmitter,[3] and a molecule involved in immune defense as well as in autoimmune-mediated tissue damage.[4] The endogenous synthesis of NO is derived from the oxidation of L-arginine to L-citrulline mediated by a family of enzymes known as nitric oxide synthease (NOS) as shown in Scheme I.[1,5,6] A couple of distinct constitutive NOS isoforms have been distinguished in the vascular endothelium[7] (eNOS, involved in the regulation of smooth muscle relaxation and blood pressure), and in the brain (nNOS, important to long-term potentiation), along with an inducible form (iNSO), which is produced by activated macrophage cells during an immune response. Despite the importance of NO, its overproduction can be hazardous to tissues because of its reactivity and free radical structure.[8,9] When this occurs, inhibitors of NOS would be important to decrease the concentration of NO in the cell. Therefore, regulation of NO concentration in tissues may have broad therapeutic applications in the treatment of human diseases such as cancers, cardiovascular, and infectious diseases.[10]

Scheme I Reaction catalyzed by nitric oxide synthase



Although the detailed mechanism of NOS is still unclear, previous studies have indicated that inhibition of NOS can be achieved with relatively simple modification of the natural substrate L-arginine. Many different inhibitors of NOS are known so far;[11] some of the earliest inhibitors included the *N*-substituted-L-arginine analogues. For example, N^G-methyl-L-arginine (NMA) and N^G-methyl-N'^G-hydroxy-L-arginine (NOHNMA) have been shown to be mechanism-based irreversible inhibitors of NOS enzyme isoform,[5b] as shown in Fig. 1. Therefore, the ready availability of various N^G-substituted-L-arginines is indispensable for investigation of mechanistic studies of this important enzymatic process. The existing preparative methods for N^G-alkyl-L-arginines, however, suffered from either low yield or low scale. In addition, it has been found recently that *N*-phenyl ring is the key component of *S*-ethyl *N*-phenylisothiourea[12] to be a potent inhibitor of both the human constitutive and inducible isoforms of NOS, but to our knowledge N^G-aryl-L-arginine has never been prepared. These considerations prompt us to develop a general and effective chemical method for preparation of N^G-alkyl and N^G-aryl-L-arginines as potential nitric oxide synthase inhibitors.

RESULTS AND DISSCUSION

The synthesis of N^G-substituted L-arginine has most

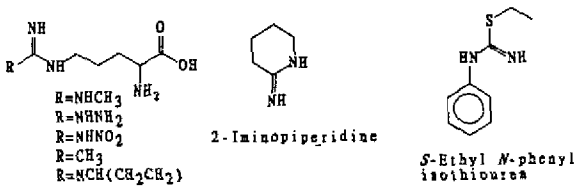
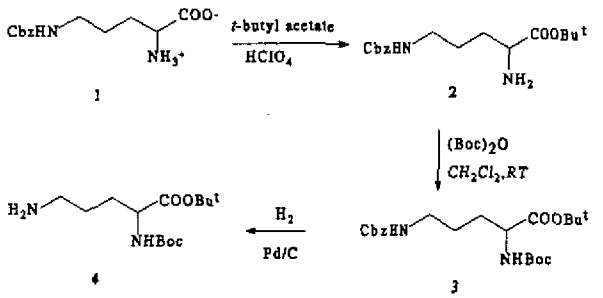


Fig. 1. Some inhibitors for nitric oxide synthase.

frequently been accomplished by the nucleophilic attack of δ-amino group of L-ornithine on the methylthiocarbonyl of a substituted pseudothiouronium salt in the presence of strong base sodium hydroxide.[13] This key reaction suffered from low yield and the risk of racemization at the α-carbon of L-ornithine.[14] In 1991, Fukuto[15] et al. used the protected N^{δ}-cyano-L-ornithine to react with hydroxylamine to generate the corresponding quanidino functionality. Subsequent acid hydrolysis obtained the final *N*-substituted L-arginine. Disadvantages of this alternative include that the synthesis is linear instead of convergent and that N^{G}-aryl-L-arginines cannot be prepared because of aryl amines' weak nucleophicility toward cyanamide.[16] Here we wish to report a general and effective procedure for synthesis of both N^{G}-alkyl and N^{G}-aryl-L-arginine derivatives with relatively high overall yield. The key step of our synthesis involved the coupling of protected L-ornithine **4** with isothiourea **7**, to give the fully protected N^{G}-substituted L-arginine derivative **8**. Subsequent deprotection of **8** in acidic condition afforded the final target compound **9** with an overall yield of more than 80%. The L-ornithine derivative **4** was prepared, as shown in Scheme II, in three steps employing a procedure in the literature:[15] Protection of the α-amino group of **1** (Sigma, St. Louis, MO) yielded its *t*-butoxycarbonyl (BOC) derivative which was esterified using *t*-butyl acetate and subsequently hydrogenated to give **4** in 70% overall yield.

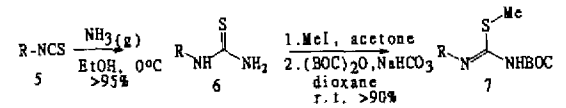
Scheme II Synthesis of N^{α}-(*tert*-butyloxycarbonyl)-L-ornithine *tert*-butyl ester **4**



Synthesis of **7** commenced with the conversion of the commercially available alkyl or aryl isothiocyanate **5** to the corresponding thiourea **6** with an excess of NH_3 in EtOH at 0 °C. Treatment of **6** with methyl iodide in acetone under reflux conditions to effect *S*-alkylation[17] was followed by isothioureido nitrogen protection with *tert*-butyl pyrocarbonate in methylene chloride at room temperature to afford **7** (Scheme III).

Replacement of the thiomethyl group of isothiourea **7**

Scheme III Preparation of alkyl and aryl isothiourea derivatives



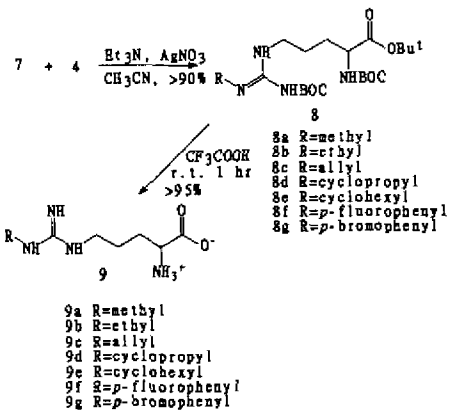
5c R=allyl
5d R=cyclopropyl
5e R=cyclohexyl
5f R=*p*-fluorophenyl
5g R=*p*-bromophenyl

6a R=methyl
6b R=ethyl
6c R=allyl
6d R=cyclopropyl
6e R=cyclohexyl
6f R=*p*-fluorophenyl
6g R=*p*-bromophenyl

7a R=methyl
7b R=ethyl
7c R=allyl
7d R=cyclopropyl
7e R=cyclohexyl
7f R=*p*-fluorophenyl
7g R=*p*-bromophenyl

with L-ornithine derivative **4** was achieved by adding a CH_3CN solution of $AgNO_3$ to a mixture of **7**, **4** and triethylamine in CH_3CN at 0 °C for 2 hours.[17] The final removal of both BOC protecting groups and hydrolysis of the *t*-butyl ester was done in one step by treating **8** with trifluoroacetic acid at room temperature for one hour to give the target compound **9** as shown below in Scheme IV.

Scheme IV Synthesis of N^{G}-alkyl, N^{G}-aryl-L-arginine derivatives



8a R=methyl
8b R=ethyl
8c R=allyl
8d R=cyclopropyl
8e R=cyclohexyl
8f R=*p*-fluorophenyl
8g R=*p*-bromophenyl

9a R=methyl
9b R=ethyl
9c R=allyl
9d R=cyclopropyl
9e R=cyclohexyl
9f R=*p*-fluorophenyl
9g R=*p*-bromophenyl

CONCLUSION

A convenient preparation of N^{G}-alkyl and N^{G}-aryl-L-arginine is reported. Compared with the previous methods, this synthetic route has the advantages of higher overall yield and no risk of losing the stereochemical integrity of the amino acid residue as well. Since the overall yield of this NOS substrate analogue synthesis is more than 80%, the ready availability of various N^{G}-substituted-L-arginines will not only facilitate the characterization of the intriguing enzymatic process catalyzed by nitric oxide synthase, but also may serve as the potential therapeutic agents for treatment of diseases mediated by excessive production of nitric oxide.

EXPERIMENTAL SECTION

General

Melting points were determined with a Mel-Temp apparatus and were uncorrected. FAB-MS and high resolution FAB-MS were measured with a JEOL JMS-SX/SX 102A spectrometer using NBA as suspensing matrix. Infrared spectra were measured with a Perkin-Elmer spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz on a Varian VXR300 spectrometer. Chemical shifts were reported in ppm on the δ scale relative to internal standard (tetramethylsilane, or appropriate solvent peaks) with coupling constants given in hertz. Flash chromatography was performed in columns of various diameters with Merck silica gel (230-400 mesh ASTM 9385 kieselgel 60H) by elution with the solvents reported. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60G-254 plates (25 mm) and developed with the solvents mentioned. TLC spots were visualized either with UV light or by dipping the plates into the staining solutions of phosphomolybdic acid (7% ethanolic solution) and then heating them. Solvents, unless otherwise specified, were reagent grade and distilled once prior to use. Methyl thiourea (**6a**) and Ethyl thiourea (**6b**) are commercially available.

General procedure for preparation of thiourea **6**: Substituted isothiocyanate **5** (4.68 mmol, purchased from Aldrich Chemical Co.) was dissolved in ethanol (20 mL). A vigorous stream of NH_3 gas was introduced while the reaction mixture was cooled in an ice-bath. After the reaction was complete in seconds, the solvent was removed by rotary evaporation. The crude product **6** was obtained quantitatively as a white solid and used without further purification in the next reaction.

Allyl Thiourea (6c)

White solid. R_f = 0.35 (50% EtOAc/hexanes). mp 74-75 °C (lit.[5c] 73-73.5 °C). ^{1}H NMR (CD_3OD) δ 5.87 (1H, bs, C*H*=CH_2), 5.25-5.10 (2H, m, CH=C*H*$_2$), 4.15-4.09 (2H, bs, C*H*$_2$CH allylic). ^{13}C NMR (CD_3OD) δ 185.0 (C=S), 135.6 (*C*H=CH_2), 116.5 (CH=*C*H$_2$), 46.5 (*N*-CH_2). FAB MS (M+1) 117 (base peak). IR (KBr) 3646, 1618, 1561, 1157 cm^{-1}.

Cyclopropyl Thiourea (6d)

White solid. R_f = 0.52 (100% EtOAc). mp 139-140 °C (lit.[5c] 142.5-143 °C). ^{1}H NMR (CD_3OD) δ 2.46 (1H, bs, CH cyclopropyl H's), 0.78 (2H, m, cyclopropyl H's), 0.58 (2H, m, cyclopropyl H's). ^{13}C NMR (CD_3OD) δ 183.5 (C=S), 25.1 (*C*H cyclopropyl), 7.3 (CH_2 cyclopropyl). FAB MS (M+1) 117 (base peak). IR (KBr) 3390, 1613, 1496, 830 cm^{-1}.

Cyclohexyl Thiourea (6e)

White solid. R_f = 0.29 (50% EtOAc/hexanes). mp 164-165 °C. ^{1}H NMR (Acetone-d_6) δ 4.02-4.00 (1H, bs, CH cyclohexyl), 1.96-1.17 (10H, m, cyclohexyl H's). ^{13}C NMR (CD_3OD) δ 184.0 (C=S), 53.9, 33.2, 26.2, 25.6 (cyclohexyl C's). FAB MS (M+1) 159 (base peak). IR (KBr) 3608, 1620, 1561, 970 cm^{-1}.

p-Fluorophenyl Thiourea (6f)

White solid. R_f = 0.42 (50% EtOAc/hexanes). mp 166-167 °C. ^{1}H NMR (CD_3OD) δ 7.33-7.30 (2H, m, Ar H's), 7.14-7.08 (2H, m, Ar H's). ^{13}C NMR (CD_3OD) δ 183.3 (C=S), 162.3 (d, J = 244.8, F-C), 135.6 (Ar-C's), 128.2 (d, J = 8.8, Ar C's), 116.9 (d, J = 23.1, Ar C's). FAB MS (M+1) 171 (base peak). IR (KBr) 3276, 1624, 1524, 828 cm^{-1}.

1-*p*-Bromophenyl 2-Thiourea (6g)

White solid. R_f = 0.44 (50% EtOAc/hexanes). mp 183-184 °C. ^{1}H NMR (CD_3OD) δ 7.50 (2H, d, J = 8.7, Ar H's), 7.30 (2H, d, J = 8.7, Ar H's). ^{13}C NMR (CD_3OD) δ 183.3 (C=S), 139.1, 133.2, 127.2, 119.8 (Ar C's). FAB MS (M+1) 231, 233 (base peak). IR (KBr) 3410, 1620, 1509, 1011 cm^{-1}.

General procedure for preparation of isothiourea **7**: To a stirred suspension of substituted 2-thiourea **6** (2.86 mmol) in 10 mL of acetone at room temperature was added dropwise 214 μL (3.43 mmol) of methyl iodide. This was heated to reflux for 1 hr and then was allowed to cool to room temperature. After acetone was removed by rotary evaporation, the residue was dissolved in dioxane (10 mL) and saturated sodium bicarbonate solution (10 mL) and *tert*-butyl pyrocarbonate (750 mg, 3.43 mmol) were added. The resulting mixture was then stirred overnight, the solvent removed by rotary evaporation, and the product purified by flash chromatography (3% EtOAc/hexanes) to give a colorless oil or white solid with the yield of more than 90%.

N-Methyl, *N'*-*t*-Butyloxycarbonyl *S*-Methylisothiourea (7a)

Colorless oil. R_f = 0.50 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 3.29 (3H, s, *N*-CH_3), 2.30 (3H, s, *S*-Me), 1.53 (9H, s, *t*-butyl). ^{13}C NMR ($CDCl_3$) δ 163.4 (carbonyl C), 153.7 (isothioureido C), 82.7 (O*C*(CH_3)$_3$), 34.3 (*N*-CH_3), 28.2 (OC(*C*H$_3$)$_3$), 14.9 (*S*-Me). The other isomer: R_f = 0.43 (25% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) δ 2.98 (3H, d, J = 5.1, *N*-CH_3), 2.48 (3H, s, *S*-Me), 1.51 (9H, s, *t*-butyl). ^{13}C NMR ($CDCl_3$) δ 163.4 (carbonyl C), 153.7

(isothioureido C), 79.2 ($OC(CH_3)_3$), 29.9 (*N*-CH_3), 28.1 ($OC(CH_3)_3$), 13.4 (*S*-Me). High-resolution FAB-MS: calcd for $C_8H_{17}N_2O_2S$ $(M+1)^+$ 204.0934, found 204.0938. IR (KBr) 3716, 1718, 1637, 855 cm^{-1}.

N-Ethyl, N′-t-Butyloxycarbonyl S-Methylisothiourea (7b)

Colorless oil. R_f = 0.56 (12.5% EtOAc/hexanes). ^1H NMR ($CDCl_3$) δ 3.35 (2H, q, *J* = 7.2, CH_2-CH_3), 2.47 (3H, s, *S*-Me), 1.51 (9H, s, *t*-butyl), 1.27 (3H, t, *J* = 7.2, CH_2-CH_3). ^{13}C NMR ($CDCl_3$) δ 173.3 (carbonyl C), 162.3 (isothioureido C), 79.1 ($OC(CH_3)_3$), 38.5 (*N*-CH_2), 28.2 ($OC(CH_3)_3$), 14.6 (*S*-Me), 13.4 (CH_2-CH_3). The other isomer: R_f = 0.52 (12.5% EtOAc/hexanes). ^1H NMR ($CDCl_3$) δ 3.79 (2H, q, *J* = 6.9, CH_2-CH_3), 2.27 (3H, s, *S*-Me), 1.48 (9H, s, *t*-butyl), 1.16 (3H, t, *J* = 6.9, CH_2CH_3). ^{13}C NMR ($CDCl_3$) δ 162.7 (carbonyl C), 153.4 (isothioureido C), 82.3 ($OC(CH_3)_3$), 42.2 (*N*-CH_3), 28.0 ($OC(CH_3)_3$), 14.8 (*S*-Me), 13.8 (CH_2CH_3). High-resolution FAB-MS: calcd for $C_9H_{19}N_2O_2S$ $(M+1)^+$ 218.1090, found 218.1086. IR (KBr) 3646, 1719, 1578, 806 cm^{-1}.

N-Allyl, N′-t-Butyloxycarbonyl S-Methylisothiourea (7c)

Colorless oil. R_f = 0.45 (12.5% EtOAc/hexanes). ^1H NMR ($CDCl_3$) δ 5.91-5.82 (1H, m, CH=CH_2), 5.32-5.20 (2H, m, CH=CH_2), 3.96 (2H, bs, *N*-CH_2), 2.47 (3H, s, *S*-Me), 1.51 (9H, s, *t*-butyl). ^{13}C NMR ($CDCl_3$) δ 173.4 (carbonyl C), 162.8 (isothioureido C), 132.4 (CH=CH_2), 117.5 (CH=CH_2), 79.2 ($OC(CH_3)_3$), 46.0 (*N*-CH_2), 28.0 ($OC(CH_3)_3$), 13.5 (*S*-Me). The other isomer: R_f = 0.52 (12.5% EtOAc/hexanes). ^1H NMR ($CDCl_3$) δ 5.92-5.83 (1H, m, CH=CH_2), 5.21-5.13 (2H, m, CH=CH_2), 4.42 (2H, bs, *N*-CH_2), 2.30 (3H, s, *S*-Me), 1.51 (9H, s, *t*-butyl). ^{13}C NMR ($CDCl_3$) δ 162.1 (carbonyl C), 153.3 (isothioureido C), 133.4 (CH=CH_2), 116.2 (CH=CH_2), 82.7 ($OC(CH_3)_3$), 49.2 (*N*-CH_2), 28.1 ($OC(CH_3)_3$), 14.8 (*S*-Me). High-resolution FAB-MS: calcd for $C_{10}H_{19}N_2O_2S$ $(M+1)^+$ 230.1090, found 230.1092. IR (KBr) 3655, 1718, 1577, 888 cm^{-1}.

N-Cyclopropyl, N′-t-Butyloxycarbonyl S-Methylisothiourea (7d)

Colorless oil. R_f = 0.46 (12.5% EtOAc/hexanes). ^1H NMR ($CDCl_3$) δ 9.75 (1H, bs, NH), 2.60 (1H, m, CH cyclopropyl), 2.45 (3H, s, *S*-Me), 1.49 (9H, s, *t*-butyl), 0.85-0.83 (2H, m, cyclopropyl), 0.71 (2H, m, cyclopropyl). ^{13}C NMR ($CDCl_3$) δ 176.2 (carbonyl C), 162.0 (isothioureido C), 79.2 ($OC(CH_3)_3$), 28.2 ($OC(CH_3)_3$), 24.6 (CH cyclopropyl), 13.6 (*S*-Me), 8.2 (CH_2 cyclopropyl). High-resolution FAB-MS: calcd for $C_{10}H_{19}N_2O_2S$ $(M+1)^+$ 230.1090, found 230.1093. IR (KBr) 3675, 1634, 1366, 891 cm^{-1}.

N-Cyclohexyl, N′-t-Butyloxycarbonyl S-Methylisothiourea (7e)

White solid. R_f = 0.50 (12.5% EtOAc/hexanes). mp 47-48 °C. ^1H NMR ($CDCl_3$) δ 9.77-9.74 (1H, bs, NH), 3.53-3.51 (1H, bs, *N*-CH), 2.45 (3H, s, *S*-Me), 1.99-1.96 (2H, m, cyclohexyl H's), 1.73-1.60 (3H, m, cyclohexyl H's), 1.49 (9H, s, *t*-butyl), 1.36-1.27 (5H, m, cyclohexyl H's). ^{13}C NMR ($CDCl_3$) δ 171.9 (carbonyl C), 162.2 (isothioureido C), 79.0 ($OC(CH_3)_3$), 53.0, 33.1, 25.2, 24.5 (cyclohexyl C's), 28.2 ($OC(CH_3)_3$), 13.5 (*S*-Me). High-resolution FAB-MS: calcd for $C_{13}H_{25}N_2O_2S$ $(M+1)^+$ 272.1560, found 272.1566. IR (KBr) 3655, 1634, 1585, 980 cm^{-1}.

N-p-Fluorophenyl, N′-t-Butyloxycarbonyl S-Methylisothiourea (7f)

White solid. R_f = 0.55 (12.5% EtOAc/hexanes). mp 74-75 °C. ^1H NMR ($CDCl_3$) δ 11.24 (1H, bs, NH), 7.28-7.22 (2H, m, Ar H's), 7.10-7.04 (2H, m, Ar H's), 2.41 (3H, s, *S*-Me), 1.55 (9H, m, *t*-butyl). ^{13}C NMR ($CDCl_3$) δ 173.5 (carbonyl C), 161.9 (d, *J* = 248.0, F-C), 152.1 (isothioureido C), 132.7, 129.0-128.5, 116.7-115.9 (Ar C's), 80.0 ($OC(CH_3)_3$), 28.1 ($OC(CH_3)_3$), 14.0 (*S*-Me). The other isomer: ^1H NMR ($CDCl_3$) δ 7.10-7.04 (2H, m, Ar H's), 6.85 (1H, bs, NH), 6.81-6.77 (2H, m, Ar H's), 2.41 (3H, s, *S*-Me), 1.44 (9H, m, *t*-butyl). ^{13}C NMR ($CDCl_3$) δ 162.1 (carbonyl C), 159.4 (d, *J* = 243.0, F-C), 150.5 (isothioureido C), 143.7, 122.3, 116.7-115.9 (Ar C's), 82.8 ($OC(CH_3)_3$), 27.8 ($OC(CH_3)_3$), 14.0 (*S*-Me). High-resolution FAB-MS: calcd for $C_{13}H_{18}FN_2O_2S$ $(M+1)^+$ 284.0819, found 284.0827. IR (KBr) 3678, 1701, 1685, 846 cm^{-1}.

N-p-Bromophenyl, N′-t-Butyloxycarbonyl S-Methylisothiourea (7g)

Colorless oil. R_f = 0.67 (12.5% EtOAc/hexanes). ^1H NMR ($CDCl_3$) δ 11.31 (1H, bs, NH), 7.50-7.47 (2H, m, Ar H's), 7.16-7.14 (2H, m, Ar H's), 2.40 (3H, s, *S*-Me), 1.54 (9H, s, *t*-butyl). ^{13}C NMR ($CDCl_3$) δ 172.6 (carbonyl C), 152.1 (isothioureido C), 146.7, 132.6, 127.9, 121.1 (Ar C's), 82.7 ($OC(CH_3)_3$), 28.2 ($OC(CH_3)_3$), 13.9 (*S*-Me). The other isomer: ^1H NMR ($CDCl_3$) δ 7.50-7.47 (2H, m, Ar H's), 6.83 (1H, bs, NH), 6.73 (2H, m, Ar H's), 2.40 (3H, s, *S*-Me), 1.45 (9H, s, *t*-butyl). ^{13}C NMR ($CDCl_3$) δ 162.0 (carbonyl C), 150.6 (isothioureido C), 135.9, 132.3, 122.7, 116.6 (Ar C's), 80.0 ($OC(CH_3)_3$), 28.0 ($OC(CH_3)_3$), 13.9 (*S*-Me). High-resolution FAB-MS: calcd for $C_{13}H_{18}BrN_2O_2S$ $(M+1)^+$ 344.0195, found 344.0192. IR (KBr) 3432, 1722, 1614, 1154 cm^{-1}.

ACKNOWLEDGMENT

The authors thank Dr. Lee Tai Liu for helpful discussions and the National Science Council of the Republic of China for financial support.

Received February 20, 1998.

Key Words

Nitric oxide; Nitric oxide synthase; L-arginine; L-citrulline; NOS inhibitor.

REFERENCES

1. Culotta, E.; Koshland, D. E. J. *Science* **1992**, *258*, 1862.
2. Kervin, J. F. J. *Ann. Rep. Med. Chem.* **1992**, *27*, 669.
3. Snyder, S. H. *Neuro.* **1992**, *8*, 3.
4. Snyder, S. H.; Bredt, D. S. *Sci. Amer.* **1992**, *May*, 68.
5. (a) Feldman, P. L. *Tetrahedron Lett.* **1991**, *32*, 875. (b) Feldman, P. L.; Griffith, O. W.; Hong, H.; Stuhr, D. J. *J. Med. Chem.* **1993**, *36*, 491. (c) Olken, N. M.; Marletta, M. A. J. *J. Med. Chem.* **1992**, *35*, 1137. (d) Wagenaar, F. L.; Kervin, Jr., J. F. *J. Org Chem.* **1993**, *58*, 4331.
6. Fukuto, J. M.; Stuehr, D. J.; Feldman, P. L.; Bova, M. P.; Wong, P. J. *J. Med. Chem.* **1993**, *36*, 2666.
7. Forstermann, U.; Pollock, J. S.; Nakane, M. *Trends Cardiovasc. Med.* **1993**, *3*, 104.
8. Moncada, S.; Palmer, R. M. J.; Higgs, E. A. *Pharmacol. Reviews* **1991**, *43*, 109.
9. Stamler, J. S.; Singel, D. J.; Loscalzo, J. *Science* **1992**, *258*, 1898.
10. (a) Lefer, A. M.; Lefer, D. J. *Drugs future* **1994**, *19(7)*, 665. (b) Porsti, I.; Paakkari, I. *Ann. Med.* **1995**, *27*, 407. (c) Moncada, S.; Higgs, E. A. *FASEB J.* **1995**, *8*, 1319.
11. (a) Kerwin, J. F.; Heller, M. *Med. Res. Rev.* **1994**, *14*, 23. (b) Marletta, M. A. *J. Med. Chem.* **1994**, *37*, 1899. (c) Moore, W. M.; Webber, R. K.; Fok, K. F.; Jerome, G. M.; Connor, J. R.; Manning, P. T.; Wyatt, P. S.; Misko, T. P.; Tjoeng, F. S.; Currie, M. G. *J. Med. Chem.* **1996**, *39*, 669. (d) Sorba, G.; Medana, C.; Fruttero, R.; Cena, C.; Stilo, A. D.; Galli, U.; Gasco, A. *J. Med. Chem.* **1997**, *40*, 463. (e) Fast, W.; Huff, M. El.; Silverman, R. B. *Bioorg Med. Chem. Lett.* **1997**, *7*, 1449.
12. Shearer, B. G.; Lee S.; Oplinger, J. A.; Frick, L. W.; Garvey, E. P.; Furfine, E. S. *J. Med. Chem.* **1997**, *40*, 1901.
13. Olken, N. M.; Marletta, M. A. *J. Med. Chem.* **1992**, *35*, 1137.
14. Ferrario, F.; Levi, S.; Sala, A.; Trupiano, F. *Synth. Commun.* **1991**, *21*, 99.
15. Wallace, G. C.; Fukuto, J. M. *J. Med. Chem.* **1991**, *34*, 1746.
16. Master thesis of Shi Shiu, July, **1997**, Department of Chemistry, Tunghai Christian University, Taichung, Taiwan, R.O.C.
17. Feldman, P. L. *Tetrahedron Letters* **1991**, *32*, 875.