Nitric Oxide Donors

For Pharmaceutical and Biological Applications

Edited by Peng George Wang, Tingwei Bill Cai, Naoyuki Taniguchi

WILEY-VCH Verlag GmbH & Co. KGaA

Nitric Oxide Donors
Edited by Peng George Wang,
Tingwei Bill Cai,
Naoyuki Taniguchi

Further Titles of Interest

O. Kayser, R. H. Müller (Eds.)

Pharmaceutical Biotechnology

Drug Discovery & Clinical Applications

2004
ISBN: 3-527-30554-8

C.-H. Wong (Ed.)

Carbohydrate-based Drug Discovery

2 Volumes

2003
ISBN: 3-527-30632-3

J.-C. Sanchez, G. L. Corthals, D. F. Hochstrasser (Eds.)

Biomedical Application of Proteomics

2004
ISBN: 3-527-30807-5

H. Buschmann, T. Christoph, E. Friderichs, C. Maul, B. Sundermann (Eds.)

Analgesics

From Chemistry and Pharmacology to Clinical Application

2002
ISBN: 3-527-30403-7

G. Müller, S. Petry (Eds.)

Lipases and Phospholipases in Drug Development

From Biochemistry to Pharmacology

2004
ISBN: 3-527-30677-3

Nitric Oxide Donors

For Pharmaceutical and Biological Applications

Edited by Peng George Wang, Tingwei Bill Cai, Naoyuki Taniguchi

WILEY-VCH Verlag GmbH & Co. KGaA

Editors

Professor Dr. Peng George Wang
876 Biological Sciences Building
484 W. 12th Avenue
The Ohio State University
Columbus, OH 43210, USA

Dr. Tingwei Bill Cai
876 Biological Sciences Building
484 W. 12th Avenue
The Ohio State University
Columbus, OH 43210, USA

Professor Dr. Naoyuki Taniguchi
Department of Biochemistry
Graduate School of Medicine
Osaka University Medical School
2-2 Yamadoka, Suita
Osaka 565-0871, Japan

All books published by Wiley-VCH are carefully produced. Nevertheless, authors, editors and publisher do not warrant the information contained in these books, including this book, to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data:
A catalogue record for this book is available from the British Library.

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at http://dnb.ddb.de

© 2005 WILEY-VCH Verlag GmbH & Co KGaA, Weinheim

Printed on acid-free and chlorine-free paper

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photoprinting, microfilm, or any other means – nor transmitted or translated into a machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

Composition Steingraeber Satztechnik GmbH, Dossenheim
Printing Strauss GmbH, Mörlenbach
Bookbinding Litges & Dopf Buchbinderei GmbH, Heppenheim

Printed in the Federal Republic of Germany
ISBN-13 978-3-527-31015-9
ISBN-10 3-527-31015-0

Contents

Part 1 Chemistry of NO Donors *1*

1 NO and NO Donors *3*
Tingwei Bill Cai, Peng George Wang, and Alvin A. Holder
1.1 Introduction to NO Biosynthesis and NO donors *3*
1.1.1 Nitric Oxide Synthases *3*
1.1.2 Chemistry of Reactive Nitrogen Species *6*
1.2 Classification of NO Donors *7*
1.3 New Classes of NO Donors under Development *9*
1.3.1 Nitroarene *9*
1.3.2 Hydroxamic Acids *10*
1.4 Development of NO-Drug Hybrid Molecules *10*
1.4.1 Nitrate Hybrid Molecules *11*
1.4.2 Furoxan Hybrid Molecules *13*
1.5 New Therapeutic Applications of NO Donors *14*
1.5.1 NO Donors against Cancer *15*
1.5.1.1 Diazeniumdiolates (NONOates) as Promising Anticancer Drugs *15*
1.5.1.2 The Synergistic Effect of NO and Anticancer Drugs *17*
1.5.1.3 NO-NSAIDs as a New Generation of Anti-tumoral Agents *17*
1.5.1.4 Other NO Donors with Anticancer Activity *18*
1.5.2 NO against Virus *19*
1.5.2.1 HIV-1 Induces NO Production *19*
1.5.2.2 Antiviral and Proviral Activity of NO *21*
1.5.3 Inhibition of Bone Resorption *22*
1.5.4 Treatment of Diabetes *23*
1.5.5 Thromboresistant Polymeric Films *23*
1.5.6 Inhibition of Cysteine Proteases *24*
1.6 Conclusion *24*
References *26*

2 Organic Nitrates and Nitrites *33*
Roger Harrison
2.1 Organic Nitrates *34*
2.1.1 Direct Chemical Reaction between Organic Nitrates and Thiols *35*
2.1.2 Glutathione-S-transferase *36*
2.1.3 Cytochrome P-450-dependent Systems *37*
2.1.4 Membrane-bound Enzyme of Vascular Smooth Muscle Cells *38*
2.1.5 Xanthine Oxidoreductase *38*
2.1.6 Mitochondrial Aldehyde Dehydrogenase *40*
2.1.7 Tolerance *42*
2.2 Organic Nitrites *44*
2.3 Conclusions *45*
References *47*

3 *N*-Nitroso Compounds *55*
Arindam Talukdar, Peng George Wang
3.1 Introduction *55*
3.2 *N*-Nitrosamines *56*
3.2.1 Synthesis of Nitrosamines *58*
3.2.2 Physical Properties and Reactions of *N*-Nitrosamines *59*
3.2.3 Structure–Activity Relationship of *N*-Nitrosamines *61*
3.2.4 Application of *N*-Nitrosamines *62*
3.3 *N*-Hydroxy-*N*-nitrosoamines *63*
3.3.1 Biologically Active *N*-Hydroxy-*N*-nitrosamine Compounds *64*
3.3.2 Synthesis of *N*-Hydroxy-*N*-nitrosamines *66*
3.3.3 Properties of *N*-Hydroxy-*N*-nitrosamines *68*
3.3.4 Reactivity of *N*-Hydroxo-*N*-nitrosamines *70*
3.4 *N*-Nitrosimines *72*
3.4.1 Mechanism of Thermal Reaction of *N*-Nitrosoimine *73*
3.4.2 Properties of *N*-Nitrosoimines *74*
3.4.3 Synthesis of *N*-Nitrosoimines *75*
3.5 *N*-Diazeniumdiolates *75*
3.5.1 Mechanism of NO Release *76*
3.5.2 Synthesis of *N*-Diazeniumdiolates *77*
3.5.2.1 Ionic Diazeniumdiolates *79*
3.5.2.2 O-derivatized Diazeniumdiolates *79*
3.5.3 Reactions of *N*-Diazeniumdiolates *79*
3.5.4 Clinical Applications *80*
3.5.4.1 Reversal of Cerebral Vasospasm *80*
3.5.4.2 Treatment of Impotency *81*
3.5.4.3 Nonthrombogenic Blood-contact Surfaces *81*
3.5.5 Future Directions *81*
References *83*

4 The Role of *S*-Nitrosothiols in the Biological Milieu *91*
Bulent Mutus
4.1 Structure and Cellular Reactivity of RSNOs *91*
4.1.1 RSNO Structure *91*
4.1.1.1 Enzymatic Consumption of RSNOs *92*
4.1.2 Formation of RSNOs in the Biological Milieu *93*
4.1.2.1 Nitrite Mediated *93*
4.1.2.2 NO Mediated *93*
4.1.2.3 NO Oxidation Products Mediated *93*
4.1.2.4 Metalloprotein Mediated *95*
4.1.2.5 Transnitrosation *98*
4.2 Postulated Physiological roles of RSNOs *99*
4.2.1 Regulation of Blood Flow by HbSNO *99*
4.2.2 Regulation of Ventilatory Response in the Brain by RSNOs *100*
4.2.3 Role of RSNOs in Platelet Function *100*
4.3 Conclusion *102*
References *103*

5 Metal–NO complexes: Structures, Syntheses, Properties and NO-releasing Mechanisms *109*
Tara P. Dasgupta, Danielle V. Aquart
5.1 Iron Complexes *110*
5.1.1 Nitroprusside *110*
5.1.2 Iron Porphyrin Nitrosyls *114*
5.1.3 Dinitrosyl Complexes (DNICs) *116*
5.1.4 Iron–Sulfur Cluster Nitrosyls *117*
5.2 Ruthenium Complexes *118*
5.3 Other Metal Nitrosyls *121*
5.4 Conclusion *122*
References *123*

6 The NO-releasing Heterocycles *131*
Alberto Gasco, Karl Schoenafinger
6.1 Heterocyclic N-oxides *131*
6.1.1 Furoxans *131*
6.1.1.1 General Properties *132*
6.1.1.2 Synthesis *134*
6.1.1.2.1 Dimerisation of nitrile oxides *134*
6.1.1.2.2 Dehydrogenation of α-dioximes (glyoximes) *135*
6.1.1.2.3 Action of nitrogen oxides on olefins *136*
6.1.1.2.4 Other methods *137*
6.1.1.3 NO-release *137*
6.1.1.4 Biological Actions *140*

6.1.1.4.1 Condensed furoxans *140*
6.1.1.4.2 Furoxan sulfones and carbonitriles *141*
6.1.1.4.3 Furoxancarboxamides *144*
6.1.1.5 NO-donor Hybrid Furoxans *145*
6.1.2 3,4-Dihydro-1,2-diazete 1,2-dioxides (1,2-diazetine 1,2-dioxides) *147*
6.1.2.1 Generalities *147*
6.1.2.2 Synthesis *148*
6.1.2.3 NO-release *149*
6.1.2.4 Biological Properties *150*
6.1.3 Other Heterocyclic N-oxides *151*
6.1.3.1 4H-pyrazol-4-one 1,2-dioxides (pyrazolone N,N-dioxides) *152*
6.1.3.2 2*H*-1,2,3-triazole 1-oxides *153*
6.1.3.3 1,2,3,4-Benzotetrazine 1,3-dioxides and 1,2,3-Benzotriazine 3-oxides *153*
6.2 Mesoionic Heterocycles *154*
6.2.1 Sydnonimines *155*
6.2.1.1 General Properties *155*
6.2.1.2 Synthesis *156*
6.2.1.3 NO-release *157*
6.2.1.4 Biological Properties *161*
6.2.2 Mesoionic Oxatriazoles *163*
6.2.2.1 Synthesis *164*
6.2.2.2 NO-release *165*
6.2.2.3 Biological Properties *167*
6.3 Other Heterocyclic Systems *168*
References *170*

7 *C*-Nitroso Compounds, Oximes, *N*-Hydroxyguanidines and *N*-Hydroxyureas *177*
S. Bruce King
7.1 Introduction *177*
7.2 *C*-Nitroso Compounds *177*
7.2.1 Alkyl and Aryl *C*-Nitroso Compounds *177*
7.2.1.1 Syntheses and Properties *177*
7.2.1.2 NO-releasing Mechanisms *178*
7.2.2 Acyl C-Nitroso Compounds *179*
7.2.2.1 Syntheses and Properties *179*
7.2.2.2 NO-releasing Mechanisms *180*
7.2.2.3 Structure–Activity Relationships *181*
7.3 Oximes *182*
7.3.1 Syntheses and Properties *182*
7.3.2 NO-releasing Mechanisms *184*
7.3.3 Structure–Activity Relationships *185*
7.4 *N*-Hydroxyguanidines *186*

7.4.1 Syntheses and Properties *186*
7.4.2 NO-releasing Mechanisms *187*
7.4.3 Structure–Activity Relationships *188*
7.5 *N*-Hydroxyureas *189*
7.5.1 Syntheses and Properties *189*
7.5.2 NO-releasing Mechanisms *191*
7.5.3 Structure–Activity Relationships *193*
References *195*

Part 2 NO Donors' Applications in Biological Research *201*

8 Vasodilators for Biological Research *203*
Anthony Robert Butler, Russell James Pearson
8.1 NO-donor Drugs for Biological Research *203*
8.2 Sodium Nitrite ($NaNO_2$) *203*
8.3 *S*-Nitrosothiols *204*
8.4 Metallic Nitrosyls *209*
8.5 Sodium Nitroprusside ($Na_2[Fe(CN)_5NO] \cdot 2H_2O$) *209*
8.6 Organic Nitrates *212*
8.7 Organic Nitrites *215*
8.8 NONOates *216*
8.9 NO Inhalation; NO Gas as an NO Donor *219*
8.10 Sydnonimines *222*
8.11 Conclusion *225*
References *226*

9 NO Donors as Antiplatelet Agents *233*
Anna Kobsar, Martin Eigenthaler
9.1 Introduction *233*
9.2 Molecular Mechanisms of NO-mediated Platelet Inhibition *233*
9.2.1 cGMP-dependent NO Signaling Mechanisms *234*
9.2.1.1 Regulation of cGMP Levels *235*
9.2.1.2 Effector Sites of cGMP *236*
9.2.1.3 cGMP-PK I Substrates in Platelets *237*
9.2.1.3.1 Inositol triphosphate (IP_3) receptor *237*
9.2.1.3.2 Rap 1b *238*
9.2.1.3.3 Vasodilator stimulated phosphoprotein (VASP) *238*
9.2.1.3.4 Heat shock protein hsp27 *239*
9.2.1.3.5 LASP *239*
9.2.1.3.6 Thromboxane A_2 (TxA_2) receptor *240*
9.2.1.3.7 Phosphodiesterase PDE5 *240*
9.2.2 cGMP-independent NO Signaling Mechanisms *240*

9.3 Effects of Different Groups of NO Donors on Platelets *241*
9.3.1 Diazeniumdiolates *241*
9.3.1.1 DEA/NO (Sodium 2-(*N*,N-diethylamino)-diazenolate-2-oxide) *241*
9.3.1.2 DETA NONOate ((*Z*)-1-[*N*-(2-Aminoethyl)-*N*-(2-ammonioethyl) amino]diazen-1-ium-1,2-diolate) *241*
9.3.1.3 MAHMA NONOate ((*Z*-1-[*N*-Methyl-*N*-[6-(*N*-methylammoniohexyl) amino]]diazen-1-ium-1,2-diolate) *242*
9.3.2 Sodium Nitroprusside (SNP) *242*
9.3.3 Molsidomine (3-Morpholino-sidnonimine; SIN-1) *242*
9.3.4 *S*-Nitrosothiols *243*
9.3.4.1 SNAP (*S*-Nitroso-*N*-acetyl-d,1-penicillamine) *243*
9.3.4.2 SNVP (*S*-Nitroso-*N*-valerylpenicillamine) *243*
9.3.4.3 GSNO (*S*-nitroso-glutatione) *243*
9.3.4.4 CysNO (*S*-Nitrosocysteine) *244*
9.3.4.5 SNAC (*S*-Nitroso-*N*-acetyl-cysteine) *244*
9.3.4.6 HomocysNO (*S*-Nitrosohomocysteine) *244*
9.3.4.7 RIG200 (*N*-(*S*-Nitroso-*N*-acetylpenicillamine)-2-amino-2-deoxy-1,3,4,6, tetra-*O*-acetyl-beta-D-glucopyranose) *244*
9.3.5 Organic Nitrates *245*
9.3.5.1 GTN (Glyceryl Trinitrate, Nitroglycerin, NTG) *245*
9.3.6 Mesoionic Oxatriazole Derivatives *245*
9.3.6.1 GEA-3162 (1,2,3,4-Oxatriazolium, 5-amino-3-(3,4-dichlorphenyl)-, cloride) *245*
9.3.6.2 GEA-3175 (1,2,3, 4-Oxatriazolium, -3-(3-chloro-2-methylphenyl)-5-[[(4-methylphenyl) sulfonyl]amino]-, Hydroxide Inner Salt) *246*
9.3.7 Other NO Donors *246*
9.3.7.1 OXINO (Sodium trioxdinitrate or Angel's Salt) *246*
9.3.7.2 B-NOD (2-Hydroxy-benzoid acid 3-nitrooxymethyl-phenyl ester) *246*
9.3.8 L-Arginine (L-Arg) *246*
9.4 Activators of Soluble Guanylyl Cyclase *247*
9.4.1 YC-1 (3-(5′-Hydroxymethyl-2′-furyl)-1-benzyl indazole) *247*
9.4.2 BAY 41-2272 (3-(4-Amino-5-cyclopropylpyrimidine-2-yl)-1-(2-fluorobenzyl)-1*H*-pyrazolo [3,4-b]pyridine) *247*
9.5 cGMP Analogs *247*
9.6 Inhaled NO and Platelet Inhibition *248*
9.7 Conclusion *248*
References *249*

10 Control of NO Production *255*
Noriko Fujiwara, Keiichiro Suzuki, Naoyuki Taniguchi
10.1 Introduction *255*
10.2 Structure of Nitric Oxide Synthase *256*

10.3 NO Formation *257*
10.4 L-Arginine and L-Arginine Derivatives *257*
10.4.1 Inhibitors *258*
10.4.2 Substrates *259*
10.5 Non-amino Acid Inhibitors and Non-amino Acid Substrates *261*
10.5.1 Guanidine *262*
10.5.1.1 Inhibitor *262*
10.5.1.2 Substrates *262*
10.5.2 Isothiourea (ITU) *265*
10.5.3 Amidine *267*
10.5.4 Cyclic Amidines are Potent iNOS Selective Inhibitors *268*
10.5.5 Indazole *269*
10.6 Inhibition of NOS Function Targeted towards Cofactors *270*
10.7 Regulators of NOS Gene Expression *270*
10.8 NO Formation by an NOS-independent Pathway *271*
10.8.1 Oxime *272*
10.8.2 Hydroxyurea *272*
10.9 Summary *273*
References *274*

Part 3 Clinical Applications of NO Donors *283*

11 Nitric Oxide Donors in Cardiovascular Disease *285*
Martin Feelisch, Joseph Loscalzo
11.1 Introduction *285*
11.2 Clinical Cardiovascular Applications of NO Donor Therapy – Past and Present *285*
11.3 Pharmacological Cardiovascular Mechanism of Action of NO Donors *288*
11.4 Clinically Available NO Donors: Structures and Mechanism of Action *290*
11.5 Nitrate Tolerance *293*
11.6 Is Nitrate Therapy Associated with Adverse Vascular Effects? *295*
11.7 Conclusions *295*
References *297*

12 Nitric Oxide Donors as Anti-platelet Agents for Thromboembolic Disorders: Clinical Status and Therapeutic Prognosis *299*
David R. Janero, David S. Garvey
12.1 Introduction *299*
12.2 Human Platelets, Thromboembolic Disorders, and NO *300*
12.3 Nitrovasodilators *307*

12.3.1 Glyceryl Trinitrate, Nitroglycerin (GTN) *307*
12.3.2 Isosorbide Dinitrate (ISDN) and Isosorbide Mononitrate (ISMN) *311*
12.3.3 Sodium Nitroprusside (SNP) *312*
12.4 Oxatriazolium NO Donors *314*
12.4.1 Sydnonimines *314*
12.5 Nitrosothiol NO Donors *316*
12.5.1 *S*-Nitroso-glutathione (GSNO) *316*
12.6 L-Arginine {*S*(+)-2-Amino-5-[(aminoiminomethyl)amino]pentanoic acid} (L-arg) *318*
12.7 NCX-4016 [2-Acetoxybenzoate 2-(1-nitroxy-methyl)-phenyl ester] *319*
12.8 Conclusion and Future Prospects *320*
References *323*

13 NO and Gene Regulation *329*
Jie Zhou, Bernhard Brüne
13.1 Formation of NO and RNI-signaling *329*
13.2 p53 Regulation under the Impact of RNI *331*
13.2.1 Basic Considerations: p53 Phosphorylation and Mdm2 Binding *331*
13.2.2 Molecular Mechanisms of RNI-evoked p53 Stabilization *332*
13.3 HIF-1α Regulation under the Impact of RNI *333*
13.3.1 Lessons from Hypoxia: Basic Considerations of HIF-1α Stability Regulation *333*
13.3.2 Stability Regulation of HIF-1α by NO/RNI in Normoxia versus Hypoxia *335*
13.4 RNI, p53 and HIF-1 in Tumor Biology *337*
13.5 Conclusions *339*
Abbreviations *341*
References *342*

14 Nitric Oxide and Central Nervous System Diseases *347*
Elizabeth Mazzio, Karam F. A. Soliman
14.1 General Overview – Gaining Control over Various NOS Enzymes that Concurrently Contribute to Degenerative CNS Diseases *347*
14.2 Signaling Controls – Neuronal NOS: TYPE-I *349*
14.2.1 Neurotransmission *349*
14.2.2 Neuronal Calcium Homeostasis *351*
14.3 Signaling Controls, Endothelial NOS: Type-3 *352*
14.3.1 EDRF/Vascular Tone *352*
14.3.2 eNOS, Cyclic AMP/GMP Regulation *353*
14.4 Signaling Controls, Inducible NOS: Type-2 *354*
14.4.1 Inflammation, Microglia and Astrocytes *354*
14.4.2 Stress Activated and Extra-cellular Kinases *355*
14.4.3 Cyclic AMP/Protein Kinase a *356*

14.4.4 Cyclic AMP–Phosphodiesterase Inhibitors *358*
14.4.5 Peroxisome Proliferator-activated Receptor-gamma *358*
14.5 The Neurotoxicity of NO *359*
14.5.1 Oxidative Stress *359*
14.5.2 Mitochondrial Impairment *361*
14.5.3 Permeability Transition Pore Complex, Apoptosis *363*
14.5.4 Excitotoxicity, Poly(ADP-ribose)-polymerase-1 *365*
References *369*

Index *385*

Preface

The discovery of the physiological and pathophysiological roles of nitric oxide (NO) during the 1980s was one of the most surprising and exciting developments in biological research. NO exhibits a broad range of biological activities. Thus, it comes as no surprise that, as far back as 1992, the editors of the journal *Science* called NO the molecule of the year, and in 1998, three scientists, R.F. Furchgott, L.J. Ignarro, and F. Murad, were awarded the Nobel Prize in physiology and medicine for their contribution to elucidating the role of nitric oxide in the functions of living organisms.

As a simple diatomic free radical, NO is generally considered to represent the biologically important form of the endothelium-derived relaxing factor (EDRF). Cellular NO is almost exclusively generated *via* the oxidation of L-arginine, which is catalyzed by nitric oxide synthetases (NOS). Under physiological conditions, NO directly activates soluble guanylate cyclase (sGC) to transform guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP), followed by kinase-mediated signal transduction. The endogenous formation of NO plays a key role in many bioregulatory systems, including smooth muscle relaxation, platelet inhibition, neurotransmission, and immune stimulation.

Due to the instability and inconvenient handling of aqueous solutions of authentic NO, there is increasing interest in using compounds capable of generating NO *in situ*. These compounds are called NO donors, or NO releasing agents. Glyceryl trinitrate (GTN) may be the most well known NO donor. Although the use of GTN for medicinal purposes dates back more than 150 years, little had been revealed about its physiological mechanism of action before the 1980s. It is well known that the epoch-making invention realized by Alfred Nobel in 1863 paved the way for controlled detonation of GTN. Therefore, when Nobel's physician recommended GTN as a treatment of his angina pectoris, Nobel wrote: "Isn't it the irony of fate that I have been prescribed N/G 1 [nitroglycerine] to be taken internally! They called it Trinitrin, so as not to scare the chemist and the public." Nobel would not have found it ironic if he had known that it was NO, released from GTN *in vivo*, that helps relieve angina.

In addition to organic nitrates, many other chemicals can be transformed into NO *in vitro* or *in vivo*. Due to the diversity of NO donor structures, the pathway for each class of compounds to generate NO could differ significantly, e.g., enzymatical vs.

non-enzymatical, reductive vs. oxidative, etc. As each class of compounds offers distinct biochemical properties, this allows us to choose a compound that best meets the demands of specific investigations.

Insufficient NO production causes serious medical problems. Many diseases such as hypertension, atherosclerosis and restenosis involve the deficiency of NO production. Therefore, a compound that can release NO under specific conditions can be used therapeutically to palliate NO underproduction. In fact, the best known NO donor, glyceryl trinitrate, has been used for over a century to relieve acute attacks of angina pectoris. In 1998, Carl Djerassi published a book entitled "NO", where he plotted the success of a biotech company producing NO donor compounds to treat male impotence. In reality, NO donor compounds have a variety of biomedical applications. Our latest search using the keyword "nitric oxide donor" at ScienceFinder revealed that there are 2,880 published research papers on NO donors. More importantly, there have been 105 US and world patents on the applications of NO donors in the treatment of cardiovascular diseases, central nervous systems diseases, diseases related to immunity, physiological disorders and many other medical situations. Besides supplementation of NO in a situation where a NO insufficiency may underlie the pathology, NO donors can also regulate NO-based physiological pathways, i.e., male erectile dysfunction, and improve drug safety and efficacy, such as gastrointestinal toxicity of non-steroidal anti-inflammatory drugs.

Since the mid-1980s, the development of new NO donors has offered several advantages over the previous NO donors, such as spontaneous releasing NO, donating NO under controlled rates, and even targeting NO to certain tissues. The current trends in NO donor development include discovery of new NO donors, finding novel applications of old NO donors, development of NO-drug hybrids and site-specific delivery of NO. Although a number of reviews and books on NO have been published, we felt that there was a need to publish a comprehensive text addressing the basic principles of all aspects of NO donors. This book is not only an informative resource for basic scientists in the NO field, but also for all clinicians and biologists interested in the applications of NO donors. This 14-chapter book is divided into three sections ranging from the basic chemistry of NO donors to clinically applied science. The first seven chapters present a review of medicinal chemistry of all classes of NO donors. The next three chapters continue to discuss the application of NO donors and NO inhibition in biological research. The final four chapters of the book address other important issues on biological functions of NO donors.

Integrating internationally recognized authors for each chapter was not an easy job. We really appreciate the help from all these hard-working authors. We are also grateful to the editors at Wiley-VCH – without their continuous support this project would never have been possible. We would like to sincerely thank faculty members, postdoctoral fellows, graduate and undergraduate students who have contributed so much in Wang's and Taniguchi's laboratories on nitric oxide research. These people are Libing Yu, Zhengmao Guo, Andrea McGill, Johnny Ramirez, Jun Li, Ming Xian, Adam Janczuk, Yongchun Hou, Vladislav Telyatnikov, Yingxin Zhang, Xuejun Wu, Alvin A. Holder, Qiang Jia, Zhong Wen, Xiaoping Tang, Xinchao Chen, Jaime Martin Franco, Mingchuan Huang, Dongning Lu, Arindam Talukdar, Noriko

Fujiwara, Satoshi Kazuma, and Yasuhide Miyamoto. P. George Wang acknowledges the continuing funding support (NIH 54074) over the past ten years from the National Institute of Health on the development of nitric oxide donors. Naoyuki Taniguchi was supported by the 21st Center of Excellence Program funded by the Ministry of Education, Culture, Sports, Science and Technology, Japan.

December 2004

Peng George Wang
Tingwei Bill Cai
Naoyuki Taniguchi

List of Contributors

Danielle V. Aquart
Department of Chemistry
University of the West Indies
Mona, Kingston 6
Jamaica

Bernhard Brüne
University of Kaiserslautern
Faculty of Biology
Department of Cell Biology
Erwin-Schroedinger-Straße 13/4
67663 Kaiserslautern
Germany

Anthony Robert Butler
University of St Andrews
Bute Medical School
The Bute Building
St Andrews
Fife KY16 9TS
Scotland
United Kingdom

Tingwei Bill Cai
The Ohio State University
Department of Chemistry
100 W 18th Ave
Columbus, OH 43210
U.S.A.

Tara P. Dasgupta
Chemistry Department
University of the West Indies
Mona Campus, Kingston 7
Jamaica

Martin Eigenthaler
Universität Würzburg
Medizinische Poliklinik
Klinikstr. 6–8
97070 Würzburg
Germany

Martin Feelisch
Boston University School of Medicine
650 Albany, X304
Boston, MA 02118
U.S.A.

Noriko Fujiwara
Department of Biochemistry
Hyogo College of Medicine
1-1 Mukogawa-cho
Nishinomiya
Hyogo 663-8501
Japan

David S. Garvey
NitroMed Inc.
125 Spring St.
Lexington, MA 02421
U.S.A.

Nitric Oxide Donors
Peng George Wang, Tingwei Bill Cai, Naoyuki Taniguchi (Eds.)
Copyright © 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
ISBN: 3-527-31015-0

Alberto Gasco
Faculty of Pharmacy
University of Turin
Via Pietro Giuria 9
10125 Turin
Italy

Roger Harrison
Department of Biology
and Biochemistry
University of Bath
Claverton Down
Bath, BA2 7AY
United Kingdom

Alvin A. Holder
Colorado State University
Department of Chemistry
Fort Collins, CO 80523
U.S.A.

David R. Janero
NitroMed Inc.
125 Spring St.
Lexington, MA 02421
U.S.A.

Kiyomi Kikugawa
School of Pharmacy
Tokyo University of Pharmacy
and Life Science
1432-1 Horinouchi
Tokyo 192-0392
Japan

S. Bruce King
Wake Forest University
Department of Chemistry
Salem Hall
Winston-Salem, NC 27109
U.S.A.

Anna Kobsar
Universität Würzburg
Institut für Klinische Biochemie
und Pathobiochemie
Josef-Schneider-Str. 2
97080 Würzburg
Germany

Joseph Loscalzo
Boston University School of Medicine
715 Albany Street, E113 (Dept. Medicine)
W507 (Whitaker CVI)
Boston, MA 02118
U.S.A.

Elisabeth Mazzio
College of Pharmacy and
Pharmaceutical Sciences
Florida A & M University
Tallahassee
FL 32307
U.S.A.

Bulent Mutus
Chemistry and Biochemistry
University of Windsor
Essex Hall, 401 Sunset Avenue
Windsor, Ontario N9B 3P4
Canada

Russell James Pearson
University of St Andrews
School of Chemistry
Purdie Building
St Andrews
Fife KY16 9ST
Scotland
United Kingdom

Karl Schoenafinger
Synthetic Medicinal Chemistry
Aventis Pharma Deutschland
Industriepark Hoechst
65926 Frankfurt am Main
Germany

Karam F. A. Soliman
College of Pharmacy
and Pharmaceutical Sciences
Florida A & M University
Tallahassee
FL 32307
U.S.A.

Keiichiro Suzuki
Department of Biochemistry
Hyogo College of Medicine
1-1 Mukogawa-cho
Nishinomiya
Hyogo 663-8501
Japan

Arindam Talukdar
The Ohio State University
Department of Chemistry
and Biochemistry
484 W 12th Avenue
Columbus, OH 43210
U.S.A.

Naoyuki Taniguchi
Department of Biochemistry
Graduate School of Medicine
Osaka University Medical School,
2-2 Yamadoka, Room B-1, Suita
Osaka 565-0871
Japan

Peng George Wang
The Ohio State University
Faculty Departments of Chemistry
and Biochemistry
484 W 12th Avenue,
Columbus, OH 43210
U.S.A.

Jie Zhou
University of Kaiserslautern
Faculty of Biology
Department of Cell Biology
Erwin Schroedinger Straße 13/4
67663 Kaiserslautern
Germany

Part 1 Chemistry of NO Donors

1
NO and NO Donors

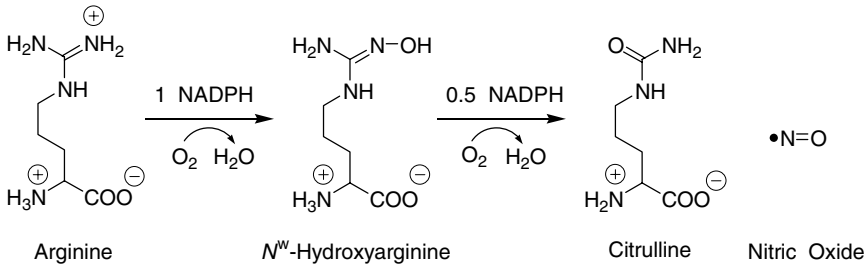
Tingwei Bill Cai, Peng George Wang, and Alvin A. Holder

Nitric oxide (NO), a magic free radical gas molecule, has been shown to be involved in numerous physiological and pathophysiological processes. Among its diverse functions, NO has been implicated in the relaxation of vascular smooth muscle, the inhibition of platelet aggregation, neurotransmission (Viagra reverses impotence by enhancing an NO-stimulated pathway), and immune regulation [1]. It was named the molecule of the year in 1992 by *Science* and was the subject of the Nobel Prize in 1998. NO has limited solubility in water (2–3 mM), and it is unstable in the presence of various oxidants. This makes it difficult to introduce as such into biological systems in a controlled or specific fashion. Consequently, the development of chemical agents that release NO is important if we are to target its bioeffector roles to specific cell types for biological and pharmacological applications. Based on our comprehensive review of NO donors [2], this chapter focuses on recent progress and current trends in NO donor development and novel applications which are not covered by the following chapters.

1.1
Introduction to NO Biosynthesis and NO donors

1.1.1
Nitric Oxide Synthases

Endogenous NO is produced almost exclusively by L-arginine catabolism to L-citrulline in a reaction catalyzed by a family of nitric oxide synthases (NOSs) [3]. In the first step, Arg is hydroxylated to an enzyme-bound intermediate N^{ω}-hydroxy-L-arginine (NHA), and 1 mol of NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) and O_2 are consumed. In the second step, NHA is oxidized to citrulline and NO, with consumption of 0.5 mol of NADPH and 1 mol of O_2 (Scheme 1.1). Oxygen activation in both steps is carried out by the enzyme-bound heme, which derives electrons from NADPH. Mammalian NOS consists of an N-terminal oxy-



Scheme 1.1 Endogenous synthesis of nitric oxide.

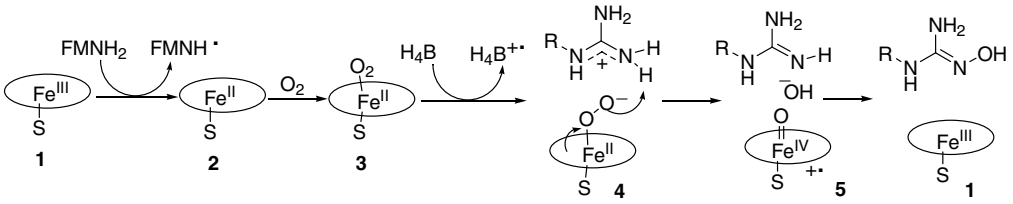
genase domain that binds iron protoporphyrin IX (heme), 6-(*R*)-tetrahydrobiopterin (H_4B) and Arg, and a C-terminal reductase domain that binds FMN (flavin mononucleotide), FAD (flavin adenine dinucleotide), and NADPH, with a calmodulin binding motif located between the two domains. To be active, two NOS polypeptides must form a homodimer. The reductase domains each transfer NADPH-derived electrons, through FAD and FMN, to the heme located in the adjacent subunit. Three distinct isoforms of NOS have been identified – neuronal, macrophage and endothelial types, and each is associated with a particular physiological process (Table 1.1). Constitutive endothelial NOS (eNOS or NOS III) regulates smooth muscle relaxation and blood pressure; constitutive neuronal NOS (nNOS or NOS I) is involved in neurotransmission and long-term potentiation; the NO produced from inducible NOS (iNOS or NOS II) in activated macrophage cells acts as a cytotoxic agent in normal immune defense against microorganisms and tumor cells. The constitutive isoforms (nNOS and eNOS) require added Ca^{2+} and calmidulin for activity and produce a relatively small amount of NO, while the inducible isoform (iNOS) has tightly bound Ca^{2+} and calmodulin, and produces a relatively large amount of NO.

Tab. 1.1: Properties of NOS isoforms.

NOS	Locations	Characteristics	Major Biological Functions
nNOS (NOS-I)	Brain, spinal cord, peripheral	Constitutive, Ca^{2+} dependent	Neuromediator
iNOS (NOS-II)	Macrophages, other tissues	Inducible, Ca^{2+} independent	Host defender, cytotoxic
eNOS (NOS-III)	Endothelium	Constitutive, Ca^{2+} dependent	Vasodilator tone modulator

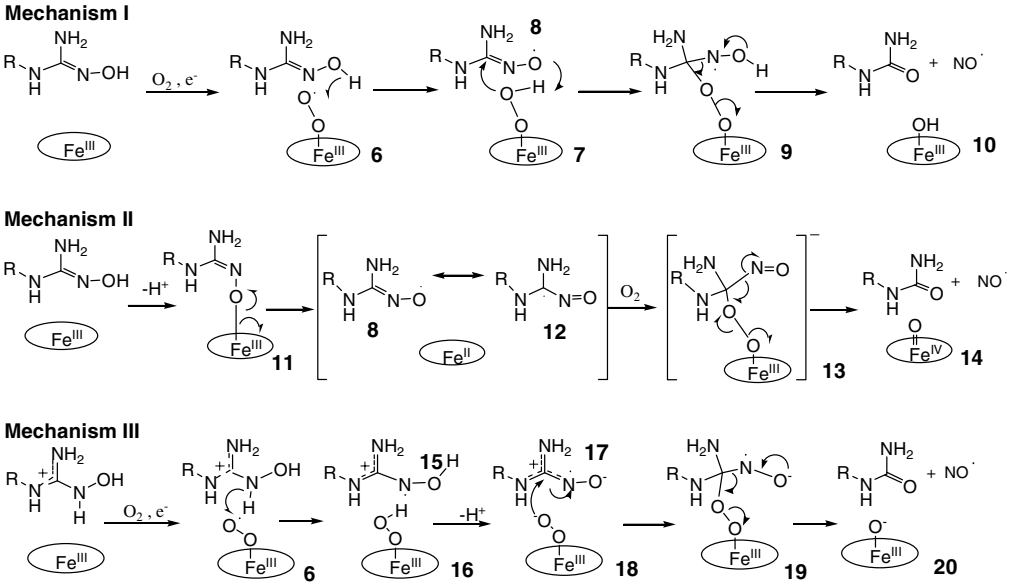
The first step of an NOS catalyzed reaction is a "classical" P450-dependent *N*-hydroxylation of a guanidine, except for the involvement of H_4B. As shown in Scheme 1.2, Fe(III)heme **1** first accepts one electron to give Fe(II)heme **2**, which binds O_2 to produce ferrous-dioxy heme **3**. The second electron from H_4B reduces **3** to peroxy-iron **4**. Arg donates a proton to **4** to facilitate O–O bond cleavage to generate an oxo-iron (IV) cation radical species **5**, which then rapidly hydroxylates the neutral guanidinium to NHA [4].

The second step of NOS oxidation is a greater challenge to enzymologists since there is no direct analogy in other systems. A variety of proposed reaction steps can be



Scheme 1.2 The first step of NOS reaction.

roughly summarized in three mechanisms (Scheme 1.3). The popular Mechanism I was proposed by Marletta and modified by Ingold and others [5, 6], a superoxoiron(III)heme intermediate **6** abstracts the hydrogen atom of the NHA to furnish an iminoxy radical **8**, which upon nucleophilic attack by the hydroperoxoiron(III)heme **7** on its carbon generates NO and citrulline. This mechanism, however, appears not to be supported by the crystal structure analysis of the NOS-NHA complex [7–9] or by a recent spectral study [10]. The second mechanism was proposed by Groves (Mechanism II), where the NOS-catalyzed aerobic oxidation of NHA occurs via a radical-type auto-oxidation process [11, 12], i.e., NHA is oxidized by the Fe(III) heme to generate an iminoxyl radical **8**, which tautomerizes to the α-nitroso radical **12**. Insertion of a dioxygen molecule between **12** and Fe(II) heme forms an energetic α-nitrosoperoxy Fe(III) heme intermediate that decomposes to generate NO [13, 14]. However, direct ligation of NHA to heme iron has been precluded by the X-ray crystallographic data [7–9]. The third mechanism, proposed by Silverman and others [15–18], mainly in-

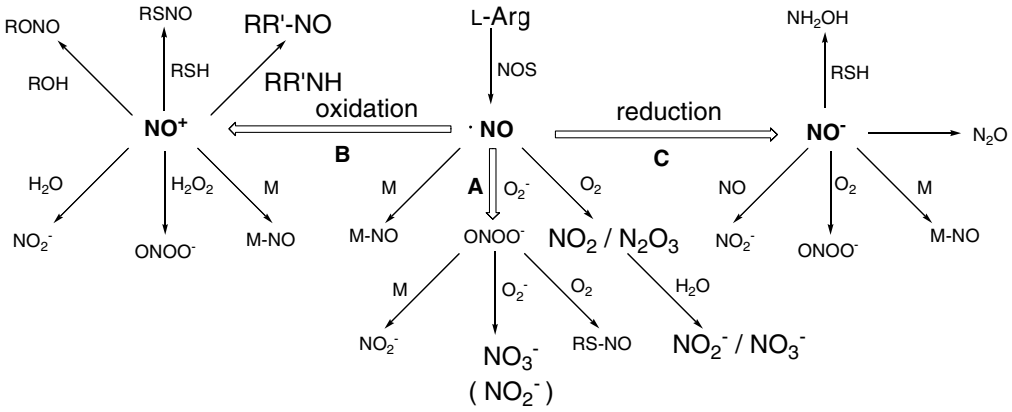


Scheme 1.3 The second step of NOS reaction.

volves the oxidation of the nitrogen on the protonated *N*-hydroxyguanidino moiety (Mechanism III). It was suggested that the initial N–H bond cleavage by superoxo-iron(III)heme **6** generates a radical cation intermediate **15**, which, upon heterolysis of the O–H bond, gives the iminoxy radical **17**. The nucleophilic attack of peroxo-iron(III)heme **18** on **17** gives an intermediate similar to **13**, which decomposes to NO and citrulline. More recently, Stuehr has emphasized the involvement of H_4B in the second step of the NOS reaction [19–21].

1.1.2 Chemistry of Reactive Nitrogen Species

One of NO's major biological actions is to activate guanylate cyclase directly to generate cyclic guanosine monophosphate (cGMP) as an intracellular second messenger, followed by kinase-mediated signal transduction. In another pathway, NO undergoes oxidation or reduction in biological systems to convert to many different reactive nitrogen species (RNS). It can react with molecular oxygen (O_2), superoxide anion ($O_2^{-\bullet}$) or transition metals (M) to produce RNS such as N_2O_3, NO_2, NO_2^-, NO_3^-, peroxynitrite ($OONO^-$), and metal-nitrosyl adducts (Route A, Scheme 1.4) [22, 23]. Among these RNS, peroxynitrite stands out as an important species [24, 25]. The reaction between NO and $O_2^{-\bullet}$ produces peroxynitrite at a diffusion controlled rate [26–28]. Peroxynitrite is a strong oxidizing and nitrating species that causes molecular damage leading to disease-causing cellular dysfunction [29, 30]. NO can also be rapidly oxidized by oxygen, superoxide or transition metals to nitrosonium (NO^+) which reacts with nucleophilic centers such as ROH, RSH and RR′NH to produce RO–NO, RS–NO or RR′N–NO, respectively (Route B, Scheme 1.4) [31, 32]. These products subsequently undergo other reactions to exhibit their biological effects. In addition, NO also undergoes a one-electron reduction to produce nitroxyl (NO^-) (Route C, Scheme 1.4). The reducing potential of this reduction is approximately +0.25 V [33]. Nitroxyl converts rapidly to N_2O under physiological conditions. Other competing reactions



Scheme 1.4 Oxidation and reduction of reactive nitrogen species.