Vitamin B_{12} and B_{12}-Proteins

Edited by
Bernhard Kräutler, Duilio Arigoni and
Bernard T. Golding

Lectures presented at the
4th European Symposium
on Vitamin B_{12} and B_{12}-Proteins

WILEY-VCH

Weinheim · Chichester · New York · Toronto · Brisbane · Singapore

This Page Intentionally Left Blank

Vitamin B_{12} and B_{12}-Proteins

Edited by
B. Kräutler,
D. Arigoni and B.T. Golding

WILEY-VCH

This Page Intentionally Left Blank

Vitamin B_{12} and B_{12}-Proteins

Edited by
Bernhard Kräutler, Duilio Arigoni and
Bernard T. Golding

Lectures presented at the
4th European Symposium
on Vitamin B_{12} and B_{12}-Proteins

WILEY-VCH

Weinheim · Chichester · New York · Toronto · Brisbane · Singapore

Prof. Dr. B. Kräutler
Leopold-Franzens-Universität
Innsbruck
Institut für Organische Chemie
Innrain 52a
A-6020 Innsbruck

Prof. Dr. D. Arigoni
ETH-Zürich
Laboratorium für
Organische Chemie
Universitätsstrasse 16
CH-8092 Zürich

Prof. Dr. B.T. Golding
Department of Chemistry
University of Newcastle
NE 17 RU Newcastle
upon Thyne

The cover picture shows a cartoon of B_{12}-dependent methionine synthase (see contribution by Drennan et al. in this book).
The picture was kindly provided by Martin Tollinger, University of Innsbruck.

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

Die Deutsche Bibliothek – CIP-Einheitsaufnahme
Vitamin B_{12} and B_{12}-proteins : lectures presented at the 4th European Symposium on Vitamin B_{12} and B_{12}-Proteins / ed. by Bernhard Kräutler ... - Weinheim ; Chichester ; New York ; Toronto ; Brisbane ; Singapore ; WILEY-VCH, 1998
ISBN 3-527-29480-5

Printing: Strauss Offsetdruck GmbH, D-69509 Mörlenbach
Bookbinding: Wilhelm Osswald & Co., D-67433 Neustadt/Weinstraße

Printed in the Federal Republic of Germany

Foreword

In an article written in the seventies, the decade of the previous B_{12} symposium, I was referring metaphorically to the endeavour of a chemical synthesis of vitamin B_{12} as an attempt to "connect X-ray island with the mainland of chemical experience". Marching step by step towards this molecular structure by chemical synthesis was meant to provide us with all that knowledge of chemical properties of the complex molecule, the X-ray structure determination of which had withheld from us. Of course, achieving a chemical synthesis of this vitamin was never expected to open a chemical route for B_{12} production - it was the extension of the frontiers of natural product synthesis of that time that was at stake.

It is true, however, that any new molecular territory discovered by the recognition of important practical consequences of its function and then chartered structurally by physical methods for further study, constitutes a challenge to the mind of the scientist the conqueror, the *homo faber*, the one who is archetypically driven to put his foot on any important newly discovered molecular territory by occupying it, that is, by making it in order to possess and explore it. This is the drive that reflects itself in the physicist's Richard Feynman dictum "What I cannot create, I do not understand" and, moreover, it is that human compulsion that lies at the heart of the inextricable interrelation between science and technology.

With the proceedings of the B_{12}-symposium of the nineties now in hand, I cannot help but be fascinated by the accounts of my colleagues summarizing their momentous achievements of the research on the biosynthesis of vitamin B_{12} during the last decade. It becomes clear to me that the metaphor referring to the conquest of "X-ray island B_{12}" has acquired a radically expanded meaning, one which could hardly have been considered before, certainly not within the bioorganic and natural product chemistry of the time. What I mean is the breathtaking potential for discovery and learning in the realm of biomolecular architecture brought about by the advent of molecular genetics, the rigorous new dimension of progress which is made possible by the judicious application of these (in the earthbound chemist's eye miraculous) methods in biosynthesis research, and, as a consequence of these developments, the exciting prospect of a totally enzymic *in vitro* synthesis of complex biomolecules, a new kind of natural product total synthesis altogether.

Vitamin B_{12}, this exceptional biomolecule with its exquisite molecular complexity, once again is paving the way towards progress by challenging and by leading researchers to move forward methodologically as well as conceptually to unprecedented levels of inquiry and experimentation. B_{12} has fulfilled this function before, with regard to its structure determination and its chemical total synthesis, and is doing it again in ongoing research on the intricate mechanistic pathways of B_{12} metabolic functions. How it has exerted this influence in the task of charting the pathway(s) of its biosynthesis is truly spectacular. That story clearly will remain one of the most extraordinary chapters in the history of the life scientist's struggle in this century to discover how life, a "chemical" life after all, makes its vital molecules.

There might even be more to it. The B_{12} molecule's exceptional structural and functional complexity, legendary as it has become to any chemist or biologist who had to deal with it, is

conjectured to contain information that refers not only to how life is operating today, but that may also bear on some aspects of life's early history. Once decoded, that information may well become part of a mosaic of circumstantial evidence that leads us to a chemical retrodiction of the evolution of some of the basic metabolic processes that we witness operating today. Such expectations are nourished and encouraged by what those recurringly dramatic discoveries on B_{12} biosynthesis - in conjunction with what has been learned about the chemistry of the type of structures involved - reveal to us. Nature evolved not one singular route to the corrin system; interestingly enough, another one which diverges relevantly from the first has been documented and, perhaps, we should not be taken by surprise if it turned out that there are more. In sharp contrast to the structural bottleneck of the conversion of aminolaevulinic acid to uroporphyrinogen, in evolving the transformation of the latter to a corrin, Nature could select from an entire library of opportunities, a virtual library, so to say, of thermodynamically and kinetically feasible transformation pathways covering a remarkably broad spectrum of structural diversity. Nature seems to have made use of that diversity when B_{12}-producing organisms were confronted with the photosynthesis-induced oxygen-crisis billion of years ago. It is to be expected that uncovering the entire spectrum of biosynthetic pathways that may still exist today in corrinoid producing anaerobic and aerobic microorganisms would teach us an extraordinary lesson on molecular evolution of biosynthetic pathways in earliest life. That is what makes vitamin B_{12} so fascinating, so incomparable, among the many other low molecular weight natural products, molecules which too are "chemically very interesting" and which too are "biologically of prime importance", yet which are "modern" in evolutionary terms. The B_{12}-structure with all its beautiful complexity is the architecturally richest, biosynthetically most elaborate and, therefore, the etiologically perhaps most informative member within the exquisite group of cofactor molecules which, according to the chemist's reasoning, reflect archaic types of molecular structure and are conjectured to have been part of the very beginning of metabolism as we know it today. Vitamin B_{12} and some of those other cofactors are not only vitamins to us today, but it also looks as though their structure types had been "vitamins" - in the most direct sense of the term- to life itself in one of the critical phases of its emergence.

Needless to say that this is a biased way of looking at vitamin B_{12}, biased through my predilection for the making of molecules and being captivated by the ways how Nature is making them. I neglect to do justice to many other development that distinguishes B_{12} research in the nineties so drastically from that in the seventies. The recent advent of B_{12}-on-protein X-ray structures is clearly the fulfilment of the dream of many. It undoubtedly marks the direction along which progress in B_{12} research will tend to take place in the near future. Vitamin B_{12}, the beautiful: Excitement will not cease !

The organizers of this marvellous symposium are to be congratulated. Special thanks must go to Bernhard Kräutler, who so brilliantly hosted the symposium in his beautiful hometown Innsbruck.

La Jolla, October 1997 Albert Eschenmoser

Preface

This volume reviews much of the current activities in the B_{12}-field, as covered in the lectures delivered at the "4th European Symposium on Vitamin B_{12} and B_{12}-Proteins", which was held at Innsbruck, Austria, in September 1996. This symposium had the difficult task of emulating the outstanding "3rd European Symposium on Vitamin B_{12} and Intrinsic Factor", held in 1979 in Zürich where all the great B_{12} names were gathered. The introductory remarks at the Zürich meeting, given by Lord Todd, looked back to the 'heyday' of B_{12} research, celebrated in the earlier two Hamburg meetings - the heroic experiments leading to the isolation and to the structure determination by X-ray analysis. One of the highlights of the Zürich meeting was the description of the completion of the ETH-Harvard total synthesis of the vitamin by Robert B. Woodward, who sadly died only 5 months after the meeting. In the time since the meeting in Zürich, the B_{12} community unfortunately also has lost some of its other prominent members, Lord Todd, Dorothy C. Hodgkin, Wilhelm Friedrich, Paul Dowd and Rolf Scheffold. We dedicate this volume to their memory.

Efforts aimed at the elucidation of the biosynthesis of B_{12}, another major topic at the Zürich meeting, have now been crowned by success and the complete pathway was presented in the opening key lectures in Innsbruck by Alan Battersby, Denis Thibaut and Ian Scott, and this problem can now be considered to be basically solved. Further highlights at the Innsbruck meeting dealt with the first X-ray crystal structures of two B_{12}-proteins, namely the B_{12}-binding domain of methionine synthetase from *Escherichia coli*, presented by Martha Ludwig and Rowena Matthews, and methylmalonyl-CoA mutase from *Propionibacterium shermanii*, described by Phil Evans and Peter Leadlay. Both crystal structures revealed as their most spectacular result the unexpected "base-off" mode of binding of the organometallic B_{12}-coenzymes. Many notable contributions and much stimulating discussions were centered on the mode of action of B_{12}, the third major theme of the Innsbruck meeting, and the contribution of B_{12} to newly recognized areas of biologically important organometallic processes. Among these contributions were lectures given by the groups of Wolfgang Buckel, Steve Ragsdale, JoAnne Stubbe and Rolf Thauer. Further important work presented at Innsbruck concerned structure, reactivity and spectroscopy of B_{12} derivatives, as well as the lecture section opened by Ebba Nexø on medical aspects of B_{12}, of B_{12}-binding proteins and of their receptors.

We have subdivided the present book into the main themes, presented at the Innsbruck symposium, i.e. biosynthesis, mode of action, structural and spectroscopic studies and clinical aspects. The manuscripts have been edited to a near common format, permitting linguistic nuances, some non-SI units and using the original figures and schemes, as supplied by the authors.

We especially thank Paula Enders of the Institute of Organic Chemistry, University of Innsbruck, not only for the very substantial help in the organisation of the symposium, but also for the invaluable assistance in the preparation of the camera ready version of this book.

For the support of the meeting by the University of Innsbruck, we would like to thank in particular its Rektor, Prof. Christian Smekal, and the many helping hands and heads from the Institute of Organic Chemistry. Among them we would like to name specifically Renate Hannak, Ernst Ellmerer-Müller, Karl-Hans Ongania and Ludwig Call.

We feel confident that the exciting new facets of B_{12} revealed at the Innsbruck meeting will stimulate experimental work to tackle the remaining fundamental questions. These concern especially the structure and function of B_{12} dependent enzymes and their associated mechanisms, the chemistry of organometallic B_{12} derivatives in a broader sense and the uptake, transport and role of B_{12} in human, mammalian and microbial metabolism. And so we look forward to the 5th European Symposium on B_{12}, where the B_{12} community will have attracted a new generation of younger and interdisciplinary researchers and at which we expect to learn about further decisive "strokes" in this area.

Innsbruck — Bernhard Kräutler
Zürich — Duilio Arigoni
Newcastle — Bernard T. Golding

September 1997

Contents

I B_{12}: An Overview 1

1. B_{12} Coenzymes, the Central Theme 3
   Bernhard Kräutler

II B_{12}: Biosynthesis 45

2. B_{12}-Biosynthesis in an Aerobic Organism: 47
   How the Pathway was Elucidated
   Alan R. Battersby

3. Vitamin B_{12} Biosynthesis in *Pseudomonas denitrificans* 63
   D. Thibaut, F. Blanche, B. Cameron, J. Crouzet, L. Debussche, E. Rémy, M. Vuilhorgne*

4. How Nature Synthesizes B_{12} Without Oxygen: 81
   Discoveries Along the Ancient, Anaerobic Pathway
   A. I. Scott

5. The Biosynthesis of Vitamin B_{12}: 101
   Assembly of the Tetrapyrrole Ring System
   Peter M. Shoolingin-Jordan

6. Investigations on the Biosynthesis of the 5,6- 119
   Dimethylbenzimidazole Moiety of Vitamin B_{12}
   Paul Renz

III B_{12}-Proteins: Enzymatic Methyltransfer 131

7. Cobalamin-Dependent Methionine Synthase 133
   from *Escherichia coli*: Structure and Reactivity
   C. L. Drennan, M. M. Dixon, D. M. Hoover, J. T. Jarrett, C. W. Goulding, R. G. Matthews, M. L. Ludwig**

8. EPR Spectroscopic Evidence That in the Energy Conserving Methyltransferase Complex from Methanogenic Archaea a Histidine Residue is Ligated to the Cobamide-Cobalt 157
*Ulrike Harms, Rudolf K. Thauer**

9. Discovery of a Biological Organometallic Reaction Sequence Involving Vitamin B_{12} 167
Stephen W. Ragsdale, Manoj Kumar, Shaying Zhao, Saurabh Menon, Javier Seravalli, Tzanko Doukov*

10. Corrinoid-Dependent Methyl Transfer Reactions in *Sporomusa ovata* 179
Erhard Stupperich, Ralph Konle, Michaela Lehle*

11. Spectroscopic and Molecular Genetic Characterization of the Two Mammalian B_{12}-Dependent Enzymes 189
Ruma Banerjee

IV B_{12}-Proteins: Enzymatic Rearrangements 199

12. A Mechanistic Overview of B_{12} Dependent Processes 201
Bernard T. Golding, Rosalind J. Anderson, Susan Ashwell, Christopher H. Edwards, Ian Garnett, Friedrich Kroll, Wolfgang Buckel*

13. Insights on the Reaction Mechanism of Methylmalonyl-CoA Mutase from the Crystal Structure 217
Philipp R. Evans, Filippo Mancia*

14. Tritium Isotope Effects and Site-Directed Mutagenesis as Probes of the Reaction Catalyzed by Methylmalonyl-CoA Mutase 227
*Nicolas H. Thomä, Thomas W. Meier, Peter F. Leadlay**

15. Mechanism of Coenzyme B_{12}-Dependent Carbon-Carbon and Carbon-Oxygen Rearrangements 237
*Harald Bothe, Gerd Bröker, Uta Müller, Iris Schall, Susanne Textor, Bernard T. Golding, Wolfgang Buckel**

16. Glutamate Mutase 253
E. Neil G. Marsh, Daniel E. Holloway, Hao-Ping Chen*

17. Isobutyryl-CoA Mutase from Streptomycetes 265
*Katja Burkhardt, Natalie Philippon, John A. Robinson**

18. Coenzyme B_{12}-Dependent Enzymes and Their Models 273
János Rétey

19. Model Studies for the Methylmalonyl-Succinyl Rearrangements 289
R. Keese, T. Darbre, Urs v. Arx, S. Müller, A. Wolleb-Gygi, D. Hirschi, V. Siljegovic, M. Pfammater, A. Amolins, T. Otten*

20. Recent Structure-Function Studies of B_{12} Coenzymes in Diol Dehydrase 303
Tetsuo Toraya

21. Adenosylcobalamin-Dependent Ribonucleotide Reductases: Still Amazing but no Longer Confusing 321
JoAnne Stubbe, Stuart Licht, Gary Gerfen, Domingos Silva, Squire Booker*

V B_{12}: Structure and Reactivity 333

22. High-Resolution Crystal Structures of Cobalamins 335
*Karl Gruber, Gerwald Jogl, Gerd Klintschar, Christoph Kratky**

23. New NMR Structural and Dynamical Probes of Organometallic B_{12} Derivatives 349
Robert Konrat, Martin Tollinger, Bernhard Kräutler*

24. FT-Raman Spectroscopy of Methyl-B_{12} and of Imidazole and Imidazolate Methylcobinamide Derivatives 369
Luigi G. Marzilli, Patricia A. Marzilli*

25. Coenzyme B_{12}-Based Chemical Precedent for Co-C Bond Homolysis and Other Key Elementary Steps 383
Richard G. Finke

26. Insight into the Mechanism of B_{12}-Dependent Enzymes: Magnetic Field Effects as a Probe of Reaction Mechanism and the Role of the Ribofuranose Ring Oxygen 403
*Ettaya Natarajan, Charles B. Grissom**

27. Cage Effects and Diastereomeric Control in the Breaking and Making of Carbon-Cobalt Bonds in Organocobalt Corrinoids 417
Kenneth. L. Brown, Lanxin Zhou, Daqing Zhao, Shifa Cheng, Xiang Zou*

VI Without B_{12} and With B_{12} ? 433

28. The Role of *S*-Adenosylmethionine As a Poor Man's Adenosylcobalamin in the Reaction of Lysine 2,3-Aminomutase 435
P. A. Frey, G. H. Reed, M. D. Ballinger, K. W. Lieder, W. Wu, C. H. Chang, V. Bandarian, F. J. Ruzicka, R. LoBrutto, H. Beinert*

29. New Structural and Biosynthetic Aspects of the Unusual Core Lipids from Archaebacteria 447
*Peter Galliker, Otto Gräther, Matthias Rümmler, Wolfgang Fitz, Duilio Arigoni**

VII B_{12} : Medical Aspects 459

30. Cobalamin Binding Proteins 461
Ebba Nexø

31. Cellular Surface Receptors Important for Vitamin B_{12} Nutrition 477
Søren K. Moestrup

32. The Intrinsic Factor-Cobalamin Receptor Expressed by Yolk Sac and Proximal Tubule Epithelial Cells is the Target of Teratogenic Antibodies 491
P. J. Verroust, E. I. Christensen S. K. Moestrup, T. G. Hammond, B. Seetharam*

33. The Synthesis and Biological Activity of Radiolabeled Cobalamin-Diethylenetriaminepentaacetate Complexes 505
H. P. C. Hogenkamp, D. A. Collins*

VIII Appendix 515

34. B_{12}-Nomenclature and a Suggested Atom-Numbering 517
Bernhard Kräutler

List of Abbreviations 523

Subject Index 529

This Page Intentionally Left Blank

List of Authors to whom Correspondence Should be Addressed

Prof. Dr. Duilio Arigoni
Laboratorium für Organische Chemie, ETH-Zürich, Universitätstrasse 16,
CH - 8092 Zurich, Switzerland

Prof. Dr. Ruma Banerjee
Institute of Agriculture and Natural Resources, Department of Biochemistry, University of Nebraska-Lincoln, East Campus, P.O. Box 830718, Lincoln, NE 68583-0718, USA

Prof. Sir Alan R. Battersby
University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge, CBZ 1EW, UK

Prof. Dr. Kenneth L. Brown
Department of Chemistry, Ohio University, Athens, OH 45701, USA

Prof. Dr. Wolfgang Buckel
Fachbereich Biologie-Mikrobiologie, Philipps-Universität Marburg, Karl-von-Frisch-Straße, D - 35032 Marburg, Germany

Prof. Dr. Philip R. Evans
MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK

Prof. Richard G. Finke
Department of Chemistry, Colorado State University, Fort Collins, CO 80523, USA

Prof. Dr. Perry A. Frey
Institute for Enzyme Research, University of Wisconsin-Madison, 1710 University Avenue, Madison, Wisconsin 53705-4098, USA

Prof. Bernard T. Golding
Department of Chemistry, University of Newcastle, Bedson Building,
Newcastle upon Thyne, NE1 7RU, UK

Prof. Dr. Charles B. Grissom
Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, USA

Prof. Harry P. C. Hogenkamp
Department of Biochemistry, University of Minnesota, 4-225 Millard Hall,
435 Delaware Street S. E., Minneapolis, Min 55455, USA

Prof. Dr. Reinhart Keese
Institut für Organische Chemie, Universität Bern, Freiestrasse 3, CH - 3012 Bern, Switzerland

Dr. Robert Konrat
Institut für Organische Chemie, Universität Innsbruck, Innrain 52a, A - 6020 Innsbruck, Austria

Prof. Dr. Bernhard Kräutler
Institut für Organische Chemie, Universität Innsbruck, Innrain 52a, A - 6020 Innsbruck, Austria

Prof. Dr. Christoph Kratky
Institut für Physikalische Chemie, Universität Graz, Heinrichstraße 28, A - 8010 Graz, Austria

Dr. Peter F. Leadlay
Department of Biochemistry, University of Cambridge, Tennis Court Road,
Cambridge CB2 1QW, UK

Prof. Dr. Martha L. Ludwig
Biophysics Research Division and Department of Biological Chemistry, Chemistry Building,
University of Michigan, 930 North University, Ann Arbor, MI 48109-1055, USA

Prof. E. Neil G. Marsh
Department of Biochemistry, University of Michigan, 940 North University, Ann Arbor,
MI 48109-1055, USA

Prof. Dr. Luigi G. Marzilli
Department of Chemistry, Emory University, 1515 Pierce Drive, Atlanta, Georgia 30322, USA

Prof. Dr. Rowena G. Matthews
Biophysics Research Division and Department of Biological Chemistry,
The University of Michigan, Ann Arbor, Michigan 48109, USA

Prof. Dr. Søren Moestrup
Institut for Medicinsk Biokemi, University of Århus, DK-8000 Århus C, Denmark

Prof. Dr. Ebba Nexø
Department of Clinical Chemistry, KH University Hospital of Århus,
DK - 8000- Århus, Denmark

Prof. Dr. Stephen W. Ragsdale
Institute of Agriculture and Natural Resources, Department of Biochemistry, University of
Nebraska-Lincoln, East Campus, P.O. Box 830718, Lincoln, NE 68583-0718, USA

Prof. Dr. Paul Renz
Institut für Biologische Chemie und Ernährungswissenschaft, Universität Hohenheim (140),
D-70593 Stuttgart, Germany

Prof. Dr. János Rétey
Institut für Organische Chemie, Lehrstuhl für Biochemie, Universität Karlsruhe, Richard-
Willstätter-Allee, Postfach 6980, D - 76131 Karslruhe, Germany

Prof. Dr. John A. Robinson
Organisch-Chemisches Institut, Universität Zürich, Winterthurerstrasse 190,
CH - 8092 Zürich, Switzerland

Prof. Dr. A. Ian Scott
Center for Biological NMR, Department of Chemistry, Texas A & M University,
College Station, Texas 77843-3255, USA

Prof. Dr. Peter M. Shoolingin-Jordan
Department of Biochemistry, School of Biological Sciences, University of Southampton, Basset Crescent East, Southampton SO9 3TU, UK

Prof. Dr. JoAnne Stubbe
Departments of Chemistry and Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Dr. Erhard Stupperich
Angewandte Mikrobiologie, Universität Ulm, D - 89069 Ulm, Germany

Prof. Dr. Rolf Thauer
Abteilung Biochemie, Max-Planck-Institut für Terrestrische Mikrobiologie, Karl-von-Frisch-Straße, D - 35043 Marburg, Germany

Dr. Denis Thibaut
Rhône-Poulenc Rorer S.A.F., 13, quai Jules Guesde - BP 14, F-94403 Vitry-sur-Seine, France

Prof. Dr. Tetsuo Toraya
Department of Chemistry, College of Liberal Arts and Sciences, Kyoto University, Sakyo-Ku, Kyoto 606, Japan

Prof. Dr. Pierre Verroust
U 64 Institut National de la Santé et de la Recherche Médicale, , 4, Rue de la Chine, F - 75970 Paris Cedex 20, France

This Page Intentionally Left Blank

I

B_{12}: An Overview

Figure I. Model of the three-dimensional structure of coenzyme B_{12}, according to X-ray analysis

1

B_{12}-Coenzymes, the Central Theme

Bernhard Kräutler

Institute of Organic Chemistry, University of Innsbruck
Innrain 52a, A-6020 Innsbruck, Austria

Summary. The B_{12}-field has experienced several truly seminal developments in the last decade, with contributions from a world-wide research effort in medical, biological and chemical laboratories. Key achievements, highlighted here and described in more detail in the following chapters, center around the marvellous work leading to the detailed elucidation of the B_{12}-biosynthesis and on the pioneering X-ray analytical investigations of B_{12}-proteins, revealing unexpected structural features of protein-bound B_{12}-coenzymes. In addition, further remarkable insights into the mechanisms of B_{12}-catalyzed and related non-B_{12} enzymatic radical reactions were gained, and novel metabolic processes were found, likely to involve B_{12}. Studies on structure, reactivity and spectroscopy continue to uncover more of the fundamental properties of B_{12}-derivatives. Investigations on B_{12}-binding proteins, their transport and interactions with their receptors in human metabolism testify to the ever increasing medical importance of B_{12}-derivatives. The B_{12}-field may be recognized as playing a central role in the newly developing, more extended area of bioorganometallic research.

1 Introduction

Close to fifty years after the first isolation of the red cobalt-complex vitamin B_{12} (**1**) as the (extrinsic) anti-pernicious anaemia factor [1,2], the symposium on "Vitamin B_{12} and B_{12}-Proteins" in September 1996 saw several remarkable forward strides towards the solution of some of the major B_{12}-"mysteries". To name the two major achievements, that could be reported: the elucidation of the biosynthetic pathway to B_{12} [3-5] and the exciting first X-ray crystal structures of B_{12}-binding proteins [6,7]. As highlights of the earlier symposia in this series, held in Hamburg (1956 and 1961) [8,9] and in Zürich (1979) [10] probably the elucidation of the enigmatic structures of vitamin B_{12} [11] and of coenzyme B_{12} (**2**) [12] and the synthetic conquest of the vitamin B_{12} structure [13,14] should be recalled.

The highly crystallizable, cyanide containing vitamin **1** (cyanocob(III)alamin, CNCbl) is a relatively inert Co(III)-complex and appears not to have a physiological function itself [15]. All the same, it is the most important commercially available form of the naturally occurring B_{12}-derivatives [15]. Other pharmaceutically relevant vitamin B_{12}-derivatives are the highly light sensitive and chemically more labile organometallic coenzyme forms, coenzyme B_{12} (**2**, 5'-deoxy-5'-adenosylcobalamin, AdoCbl) and methylcob(III)alamin (**3**, MeCbl), as well as the "inorganic" B_{12}-derivatives aquocob(III)alamin (as chloride $\mathbf{4}^+.Cl^-$, $H_2OCbl.Cl$) and hydroxocob(III)alamin (**5**, HOCbl) (see Figure 1).

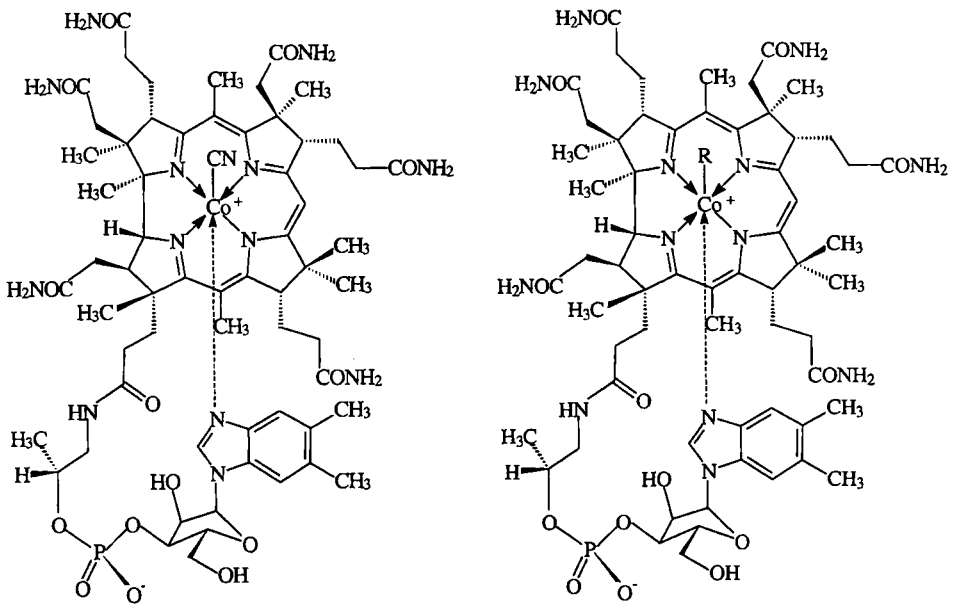
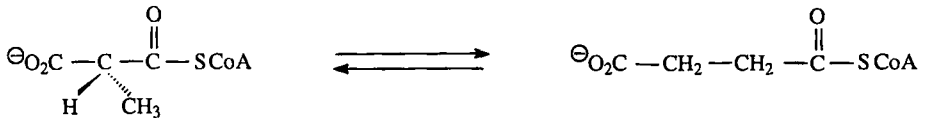


Figure 1. Structural formulae. Left: vitamin B_{12} (**1**, CNCbl); right: coenzyme B_{12} (**2**, R = 5'-deoxy-5'-adenosyl, AdoCbl), methylcobalamin (**3**, R = methyl, MeCbl), aquocobalamin cation ($\mathbf{4}^+$, R = H_2O^+, H_2OCbl^+), hydroxocobalamin (**5**, R = HO, HOCbl).

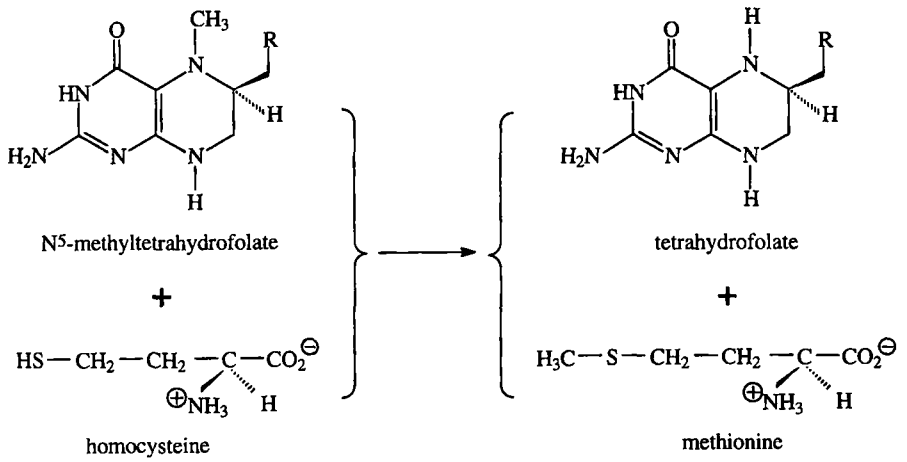
As can be gathered from many of the contributions in this book and to the now 15 year old review, " B_{12}" (edited by Dolphin) [16], the physiological roles of vitamin B_{12}-derivatives are intimately connected with their function as cofactors in enzymatic reactions. Their abilities to bind to proteins and subsequently interact with substrate molecules are questions of central importance, as are the ways in which vitamin B_{12}-forms are made available to living organisms (by their own biosynthesis [3-5,18] or else, via uptake, transport and storage [17]). The human well-being depends upon a regular supply of some of the cobalamins listed above: the B_{12}-coenzymes **2** and **3** are indispensable for human metabolism, in which the "coenzyme" **2**

cocatalyzes the enzymatic rearrangement of methylmalonyl-coenzyme A to succinyl-coenzyme A [19], while methylcobalamin (**3**) acts as cofactor in the enzyme catalyzed methylation at the sulfur of homocysteine using a methyl group from N^5-methyltetrahydrofolate, which leads to tetrahydrofolate and methionine [20] (see Scheme 1).

The coenzyme-B_{12}-catalyzed (*R*)-methylmalonyl-CoA/succinyl-CoA rearrangement:



The methylcobalamin-catalyzed methyl group transfer in methionine synthase:

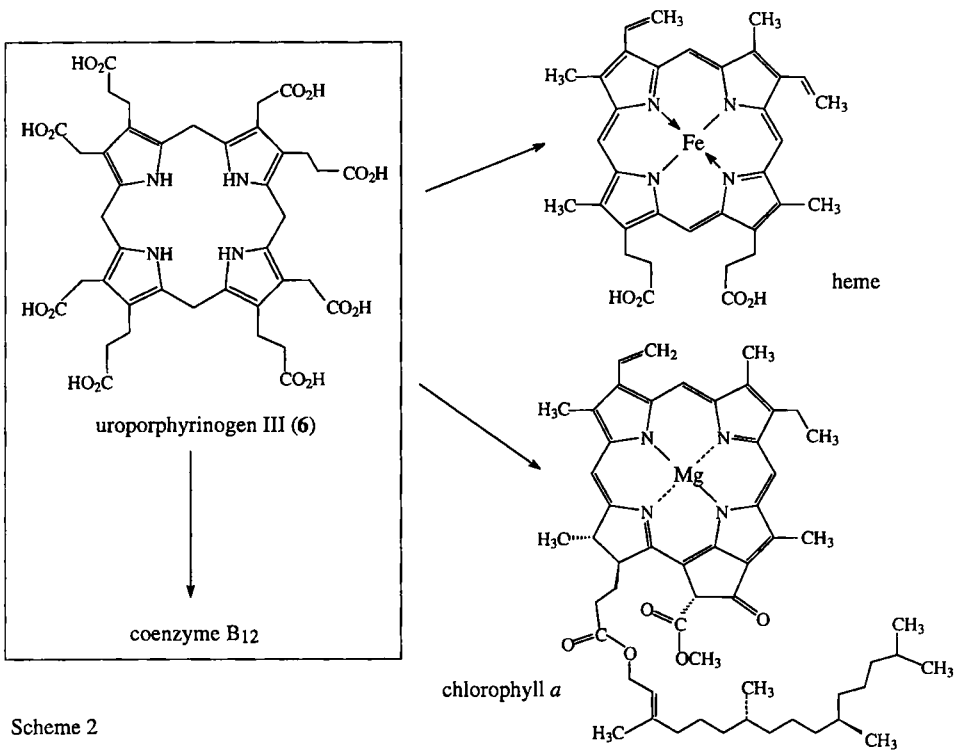


Scheme 1

The world's supply with B_{12}-derivatives depends exclusively on the activity of microorganisms, either in their natural environment, or biotechnologically exploited for the purpose of the pharmaceutically and agrotechnologically indispensable B_{12}-production. In fact, it is in the broad range of microorganisms, that B_{12}-derivatives may occupy a metabolically central position, such as in the remarkable organometallic pathway of CO_2-fixation [21]. Quite clearly, the newly developing area of bioorganometallic research nowadays still is pioneered by studies in the B_{12}-field.

2 B_{12}-Biosynthesis

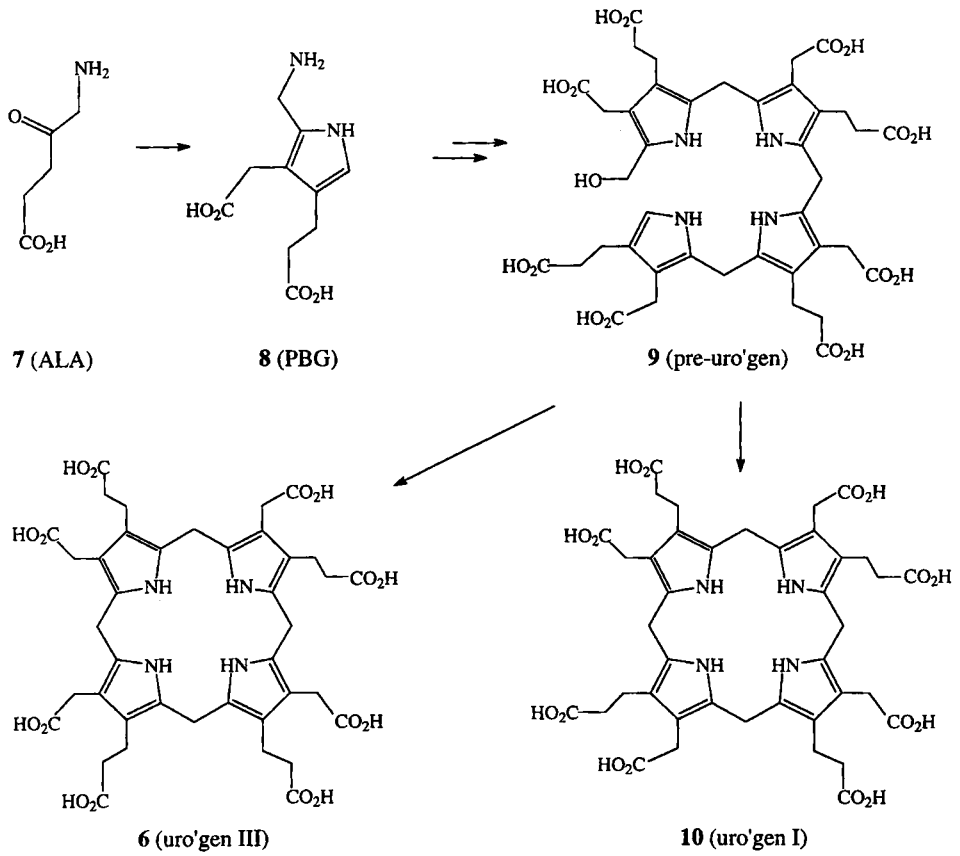
The total (non-enzymatic) synthesis of vitamin B_{12} (**1**), a unique experimental and intellectual effort [13,14], was accomplished by the time of the B_{12}-Symposium in 1979 [10]. This work has not resulted in an economical method for the nonbiological synthesis of the important cobalt-complex **1**. However, together with further elegant synthetic studies on potentially biomimetic ways to the B_{12}-structure from Eschenmoser's laboratory [22], it gave insights into the chemical reactivity of relevant porphinoid and corrinoid complexes, and pointed to the inherent tendency of the basic moieties of the B_{12}-structure to self-assemble under (proper) nonenzymatic conditions [22]. For an era of nearly two decades, these studies provided valid abiological parallels for the earlier biosynthetic stages from uroporphyrinogen III (**6**) to the corrin ligand of B_{12} and also gave guiding lines for investigations on the later parts of the B_{12}-biosynthesis. However, as reviewed in the reports of Battersby [3], Thibaut [4] and Scott [5] given in this book, our understanding of Nature's intricate paths from the porphyrinogen **6** to the corrin ligand of B_{12} has undergone a spectacular deepening in these last years and the biosynthetic pathways to the B_{12}-structure, experimentally explored so far, have been shown to deviate dramatically from those considered earlier [22-24]. This is particularly so with respect to the way in which the key step of the B_{12}-biosynthesis is accomplished, a ring-contraction from a porphinoid to a corrinoid macrocycle.



Scheme 2

2.1 Tetrapyrrole-Assembly

According to earlier investigations [25], Nature's path(s) to the B_{12}-structure first pass(es) through the stages common to the biosynthesis of all porphinoid natural products (see Scheme 2). It starts with the five-carbon unit δ-aminolaevulinic acid (**7**, ALA)), out of which a metal catalyzed enzymatic dimerization (and condensation) furnishes the functionalized pyrrole porphobilinogen (**8**, PBG) [25,26]. A remarkable enzyme catalyzed multistep deaminating tetrameroidization of PBG, delineated by P. M. Shoolingin-Jordan [27], then furnishes the linear tetrapyrrole pre-uroporphyrinogen (**9**) [25,27]. This hydroxymethylbilane would spontaneously cyclize to uroporphyrinogen I (**10**) [27,28], but, by rapid intervention of uroporphyrinogen III synthase, the cyclization of **9** is switched to uroporphyrinogen III (**6**) via a hypothetical spiro-intermediate (see Scheme 3 [27,28]). The programmed biological synthesis of **6**,and not that of the more symmetrical and biosynthetically obviously less complex **10**, has been proposed to be a sign of the pre-enzymatic origin of the natural porphinoids [22, 29].

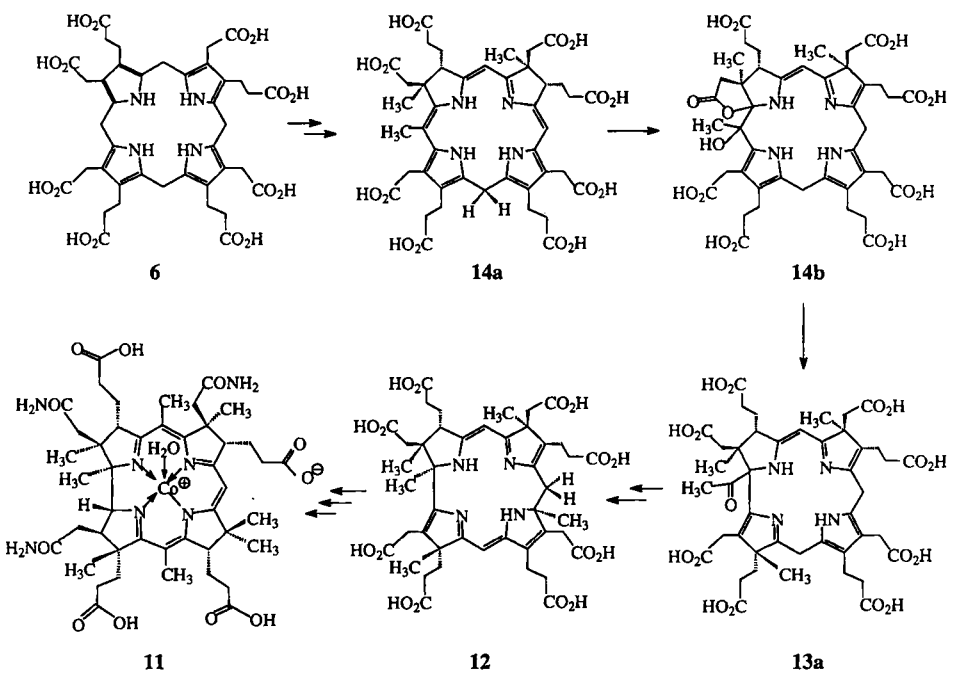


Scheme 3

2.2 Access to the Corrin Ligand

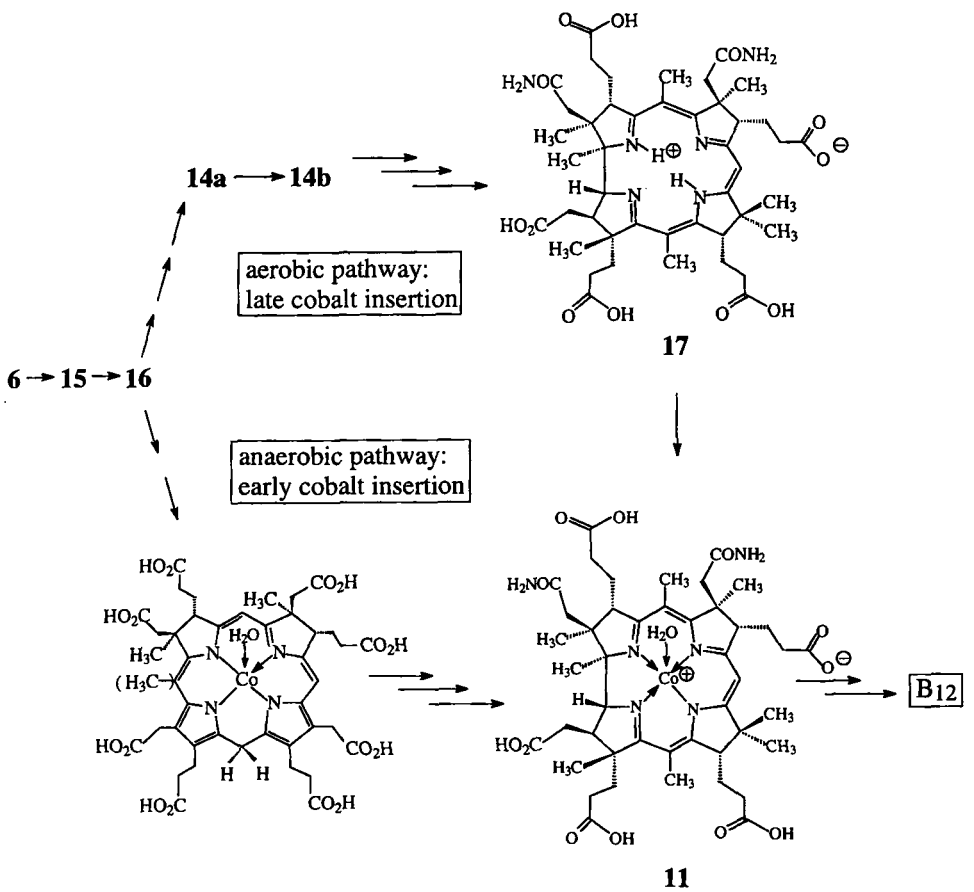
Methylation of the hexahydroporphinoid tetrapyrrole uroporphyrinogen III (**6**) with methyl groups from *S*-adenosylmethionine (SAM) at two pyrrolic β-carbons then separates off the B_{12}-biosynthesis-branch from those towards heme and the chlorophylls [25]. A total of eight methyl groups from SAM are incorporated during the sequence from uroporphyrinogen III (**6**) to the B_{12}-biosynthesis intermediate cobyrinic acid-*a*,*c*-diamide (**11**). One of them, which is incorporated at the crucial "western" meso-position, is lost again together with the excised meso-carbon [29] and indeed is believed to be placed there only to assist in the ring-contraction step (see below and [3-5]).

Remarkably, unlike the (oxidative) biosynthetic transformation of **6** to heme or chlorophyll, the path from **6** to the B_{12}-corrin ligand can be formulated without a formal (overall) redox change [22]. From this analysis, model considerations evolved, according to which also the individual steps of the B_{12}-biosynthesis would proceed without changes in the formal redox state of the tetrapyrroles involved [22].



Scheme 4

The detection and determination of the structure of "precorrin 6A" (**12**) [30] indicated a novel turn of the B_{12}-biosynthesis path (in the aerobe *Pseudomonas denitrificans*). The latter's unexpected "complexity" was further manifested in early 1993, when the structure of "Factor IV" (**13b**), a tetramethylated and (already) ring-contracted (corrinoid) oxidation product of "precorrin 4" (**13a**, a biosynthetic precursor of vitamin B_{12} from *Ps. denitrificans*) [3,4,31] was reported [32]. This unanticipated structural finding and that concerning "precorrin 6A" (**12**) [3-4,30] was soon consolidated mechanistically by the isolation and determination of the structure of the enzymatic oxidation product "precorrin 3B" (**14b**, see Scheme 4 [3-5,33]). Indeed, after the structure of **13b** was revealed and from a "chemical point of view" [34], the operation of an oxidation preceding the ring-contraction step in the B_{12}-biosynthesis appeared to make sense, as it would adjust the reactivity at the crucial "western" meso-position of the hexahydroporphinoid "precorrin 3A" (**14a**) for the incorporation of oxygen.



Scheme 5

As delineated in the following chapters by Battersby [3], Thibaut [4] and Scott [5], the biological and chemical investigations on B_{12}-biosynthesis have in fact diverged meanwhile, into studies on the aerobe *Ps. denitrificans* and (more recently also) on the anaerobic microorganism *Propionibacterium shermanii*. From this work, detailed knowledge indeed on two fairly distinct basic pathways (rather than a single one) from **6** to the highly functionalized corrinoid B_{12}-precursor cobyrinic acid-*a*,*c*-diamide (**11**) has evolved (see Scheme 5) [3-5]:
Both paths start with the hexahydroporphinoids precorrin-1 (**15**) and precorrin-2 (**16**). The anaerobic branch then passes through the incorporation of a cobalt-ion into the porphinoid macrocycle, while the aerobic branch proceeds without cobalt [3-5]. The intriguing difference between the two pathways is apparently caused by the ways in which the now crucial two-electron oxidation can be achieved to prepare for the remarkable enzyme catalyzed contraction to a corrinoid. In the aerobe *Ps. denitrificans* molecular oxygen has been recognized to play the part of the oxygenating agent in this step [3-5]. In the anaerobic path, studied with *P. shermanii*, the observed early biosynthetic incorporation of the cobalt-ion now appears to be required, as the redox-active metal-ion may take up a role in mediating the crucial early two-electron oxidation of the macrocycle [5].

2.3 Completion of the B_{12}-Structure

Interestingly, the two paths of the B_{12}-biosynthesis merge at the stage of the cobyrinic acid-*a*,*c*-diamide (**11**), formed by cobalt-incorporation into hydrogenobyrinic acid-*a*,*c*-diamide (**17**) in the aerobic path [3,4]. The presence of the *c*-acetamide group in **17** and its tendency to reversibly form the lactam **18** by (base catalyzed) addition of the *c*-acetamide group to the corrin chromophore [35] may indeed be specifically relevant for the enzymatic metal incorporation [36]: By the reversible formation of the lactam function, the corrin chromophore would be interrupted in **18** and a kink would be introduced in the corrin ligand, by which the metal incorporation may be facilitated. This may then critically assist the enzymatic cobalt incorporation [4,36], presumed to be inherently slow with an intact corrin ligand, as judged from the notorious difficulty of chemically demetallating cobalt-corrins [37].

The later steps of the B_{12}-biosynthesis in the aerobe *Ps. denitrificans* actually pass through the stage of coenzyme B_{12} (**2**) and require first the 5'-adenosylation of the central cobalt-ion of **11** giving Coβ-5'-adenosyl-cobyrinate-*a*,*c*-diamide (**19a**), followed by further amidation of side chains and the biosynthetic build-up of the nucleotide loop, via 5'-adenosylcobinamide (**19b**, AdoCbi) (see Scheme 6) [4,38]. The elucidation of the biosynthetic origin of the heterocyclic pseudo-nucleotide bases of some "complete" corrins (i.e., those, that carry an intramolecularly coordinating nucleotide function) also has revealed some unexpected turns, as reported by Renz in this book [18a] for the case of the 5',6'-dimethylbenzimidazole (DBI) base of vitamin B_{12} (**1**).

The availability of all crucial B_{12}-biosynthetic enzymes in overexpressed form and the knowledge of their operation has recently enabled Scott and coworkers to achieve a remarkable