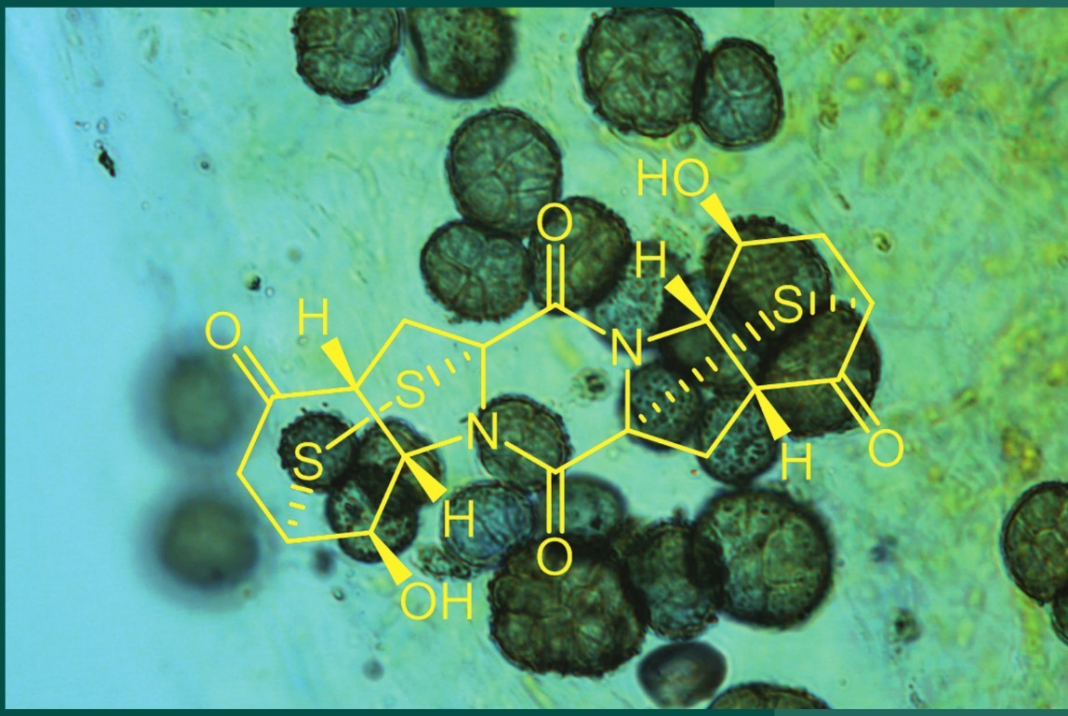
PROGRESS IN THE CHEMISTRY OF ORGANIC NATURAL PRODUCTS

97

S. Bräse, F. Gläser, C.S. Kramer,
S. Lindner, A.M. Linsenmeier, K.-S. Masters,
A.C. Meister, B.M. Ruff, and S. Zhong

The Chemistry of Mycotoxins



Springer

Progress in the Chemistry
of Organic Natural Products

Founded by L. Zechmeister

97

Progress in the Chemistry of Organic Natural Products

The Chemistry of Mycotoxins

Authors:
S. Bräse
F. Gläser
C.S. Kramer
S. Lindner
A.M. Linsenmeier
K.-S. Masters
A.C. Meister
B.M. Ruff
S. Zhong

Springer

Prof. A. Douglas Kinghorn, College of Pharmacy,
Ohio State University, Columbus, OH, USA

em. Univ.-Prof. Dr. H. Falk, Institut für Organische Chemie,
Johannes-Kepler-Universität, Linz, Austria

Prof. Dr. J. Kobayashi, Graduate School of Pharmaceutical Sciences,
Hokkaido University, Sapporo, Japan

ISSN 2191-7043 ISSN 2192-4309 (electronic)
ISBN 978-3-7091-1311-0 ISBN 978-3-7091-1312-7 (eBook)
DOI 10.1007/978-3-7091-1312-7
Springer Wien Heidelberg New York Dordrecht London

Library of Congress Control Number: 2012951144

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Contents

Listed in PubMed

List of Contributors

Stefan Bräse Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry & Institute of Toxicology and Genetics, 76131 Karlsruhe, Germany, braese@kit.edu; www.ioc.kit.edu/braese

Franziska Gläser Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry, 76131 Karlsruhe, Germany, franziska.glaeser@kit.edu

Carsten S. Kramer Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry, 76131 Karlsruhe, Germany, carsten.kramer@kit.edu

Stephanie Lindner Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry, 76131 Karlsruhe, Germany, stephanie.lindner@kit.edu

Anna M. Linsenmeier Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry, 76131 Karlsruhe, Germany, anna.linsenmeier@kit.edu

Kye-Simeon Masters Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry, 76131 Karlsruhe, Germany, kye.masters@kit.edu

Anne C. Meister Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry, 76131 Karlsruhe, Germany, anne.meister@kit.edu

Bettina M. Ruff Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry, 76131 Karlsruhe, Germany, bettina.ruff@kit.edu

Sabilla Zhong Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry, 76131 Karlsruhe, Germany, sabilla.zhong@kit.edu

About the Authors

Stefan Bräse studied in Göttingen, Bangor (UK), and Marseille (France) and received his Ph.D. in 1995, after working with Armin de Meijere in Göttingen. After post-doctoral appointments at Uppsala University (with Jan E. Bäckvall) and The Scripps Research Institute (with K. C. Nicolaou), he began his independent research career at the RWTH Aachen in 1997 (with Dieter Enders). In 2001, he finished his Habilitation and moved to the University of Bonn as professor for organic chemistry. Since 2003, he has been full professor at the Karlsruhe Institute of Technology in Germany. His research interests include methods in drug discovery (including drug delivery), combinatorial chemistry towards the synthesis of biologically active compounds, total synthesis of natural products, and nanotechnology.

Franziska Gläser, born in 1986 in Mannheim-Neckarau, studied in the University of Karlsruhe (now Karlsruhe Institute of Technology) and successfully completed her diploma project with the title "Methods for the synthesis of α,β-unsaturated aldehyde systems towards the total synthesis of blennolide D" in 2011. In the same year she began her Ph.D. thesis towards the total syntheses of natural products, under the supervision of Prof. Dr. Stefan Bräse.

Carsten S. Kramer studied biomedical chemistry and molecular biology at the University of Mainz. After finishing his diploma thesis on Aza-Claisen rearrangements and the use of microreactors in asymmetric synthesis (with U. Nubbemeyer), he was awarded the Join-the-Best-Scholarship (with the Helmholtz association as partner company), which funded his research in biophysics and cell biology at the German Cancer Research Centre and at the NIH (USA) (with J. Lippincott-Schwartz). As a Kekulé fellow, Carsten started his doctoral thesis focused on total synthesis at ETH Zurich and pursued his thesis with S. Bräse at the Karlsruhe Institute of Technology. Carsten's personal interests are total synthesis, medicine, live cell imaging, and business consulting.

Stephanie Lindner was born in 1985. She studied chemistry at the University of Karlsruhe (now Karlsruhe Institute of Technology (KIT)) and completed her diploma thesis with the title "Studies towards the total synthesis of parnafungins" in January 2011. Subsequently, she started her Ph.D. studies at the Karlsruhe Institute of Technology under the supervision of Prof. Dr. Stefan Bräse. Her scientific work focuses on the total synthesis of natural products.

Anna M. Linsenmeier, born in 1985, studied chemistry at the University of Karlsruhe (TU). She wrote her diploma thesis in the research group of Prof. Dr. Stefan Bräse, where she subsequently started her Ph.D. in 2009. After a research internship at the University of Queensland in Brisbane under the supervision of Dr. Craig M. Williams, she finished her Ph.D. at the Karlsruhe Institute of Technology (KIT) in the group of Prof. Bräse in 2012.

Kye-Simeon Masters was born in Kyogle, northern New South Wales on the summer solstice, 1979. He was greatly interested in science, the visual arts, and literature during his early schooling. An investigation into the effects of potassium permanganate on some plants in his mother's garden sparked a love for chemistry. He earned a Bachelor of Science from the Australian National University in 2002, and completed both an honors year (2004) and doctorate (2007) in total synthesis with Prof. Bernard Flynn at Monash University. A postdoctoral year followed with Prof. Bert Maes in Antwerp (2008–2009). He continued his postdoctoral research in the laboratory of Prof. Stefan Bräse with an Alexander von Humboldt Fellowship (2010–2011). His research interests are focused on natural product synthesis and innovations in transition metal catalysis.

Anne C. Meister studied chemistry at the University of Karlsruhe (now Karlsruhe Institute of Technology (KIT)) from 2005 to 2010. She completed her diploma thesis with the title "Synthesis of 4-hydroxy-5-methylcyclohex-2-enones as building blocks for the total synthesis of secalonic acids" in the group of Prof. Stefan Bräse in Karlsruhe. Since March 2010, she has been working on her Ph.D. thesis ("total synthesis of secalonic acids") in the same group.

Bettina M. Ruff was born in 1983. She studied biomedical chemistry at the University of Mainz and finished her diploma thesis ("Synthesis and testing of angiogenesis inhibitors") in the group of Prof. Gerd Dannhardt at the Institute of Pharmacy in 2008. Then, she moved to the Karlsruhe Institute of Technology (KIT) to work on her Ph.D. thesis in the group of Prof. Stefan Bräse. In 2010, she spent 6 months at the Massachusetts Institute of Technology (MIT), and received her Ph.D. ("Chemical and biochemical methods for the stereoselective synthesis of complex natural products") in December 2011 from KIT. Since 2012, she has been working with the pharmaceutical company Hoffmann-La Roche Ltd., in Basel.

Sabilla Zhong was born in 1987. She studied chemistry at the Karlsruhe Institute of Technology. In April 2011, she received her diploma degree by working on the synthesis of functionalized hexahydroindoles. In the same year, she started her Ph.D. studies at the Karlsruhe Institute of Technology with Prof. Dr. Stefan Bräse. Her scientific work focuses on the total synthesis of (thio)diketopiperazine natural products and their biological evaluation.

1 Introduction

Mycotoxins – from the Greek μύκης (*mykes*, *mukos*) "fungus" and the Latin *toxicum* "poison" – are a large and growing family of secondary metabolites and hence natural products produced by fungi, in particular by molds (*1*). It is estimated that well over 1,000 mycotoxins have been isolated and characterized so far, but this number will increase over the next few decades due the availability of more specialized analytical tools and the increasing number of fungi being isolated. However, the most important classes of fungi responsible for these compounds are *Alternaria*, *Aspergillus* (multiple forms), *Penicillium*, and *Stachybotrys*. The biological activity of mycotoxins ranges from weak and/or sometimes positive effects such as antibacterial activity (*e.g.* penicillin derivatives derived from *Penicillium* strains) to strong mutagenic (*e.g.* aflatoxins, patulin), carcinogenic (*e.g.* aflatoxins), teratogenic, neurotoxic (*e.g.* ochratoxins), nephrotoxic (*e.g.* fumonisins, citrinin), hepatotoxic, and immunotoxic (*e.g.* ochratoxins, diketopiperazines) activities (*1*, *2*), which are discussed in detail in this volume.

The hazardous nature of mycotoxins was first associated with a disease (mycoroxicosis) in the mid-1950s (*3*), however, mycotoxin-associated diseases have been known for centuries. For example, aflatoxin was isolated and identified in 1961, following a 1960 incident in which 100,000 turkey poults in the British Isles died from eating feed containing contaminated peanut meal (*3*).

Currently, many laboratories around the world have specialized in the detection of mycotoxins (*4*) in food products and contaminated housing supply materials (*5*). A large number of review articles, books, and book chapters have appeared on this topic in the last 50 years.

In this volume, we will focus on the most important classes of mycotoxins and discuss advances in their chemistry over the last ten years. In each section, the individual biological impact will be discussed. The chapters have been arranged according to mycotoxin class (*e.g.* aflatoxins) and/or structural classes (*e.g.* resorcylic acid lactones (*6*), diketopiperazines (*7*, *8*)). The biological aspects will be treated only in brief (*9*). For a recent, comprehensive treatise of mycotoxin chemistry, we refer the reader to a major review (*10*).

S. Bräse *et al.*, *The Chemistry of Mycotoxins*, Progress in the Chemistry of Organic Natural Products, Vol. 97, DOI 10.1007/978-3-7091-1312-7_1,

2 Aflatoxins

The aflatoxins were discovered in the 1960s, when they were identified as toxic compounds of the fungus *Aspergillus flavus*, which is shown in Fig. 2.1 (*11*, *12*).

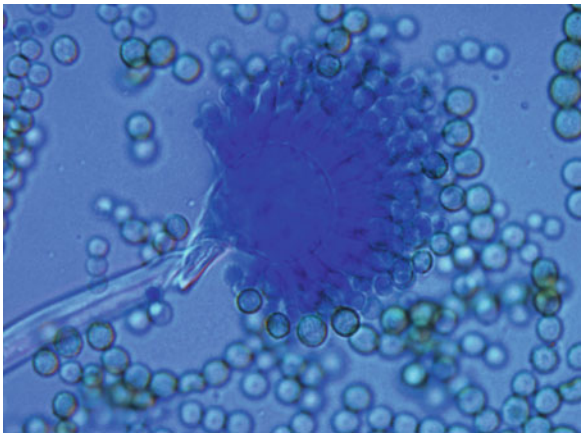
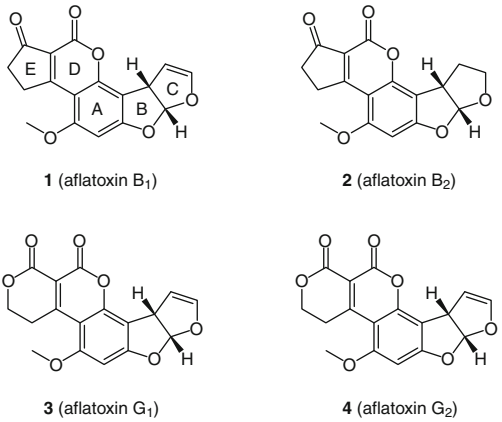


Fig. 2.1 *Aspergillus flavus* spores as seen under the light microscope under 600-fold magnification

This fungus was found in ground nut meal, which had been fed to different farm animals. Due to this contamination, 100.000 turkeys died in 1960 in Britain of the so-called "Turkey-X disease" (*13*). Later, the aflatoxins were also found in other *Aspergillus* species and in some *Penicillium* fungi. The name "aflatoxin" is an abbreviation of *Aspergillus flavus* toxins (*14*). Up to the present, the aflatoxins are among the most acutely toxic and carcinogenic compounds known (*13*). Although most countries in the world now have limitations for the maximum tolerated levels of aflatoxins in food, contamination by these compounds is still a problem (*15*). Aflatoxins are found regularly in different foods, especially the milk of cows, which gets intoxicated by affected animal feed (*13*, *15*, *16*).

S. Bräse *et al.*, *The Chemistry of Mycotoxins*, Progress in the Chemistry of Organic Natural Products, Vol. 97, DOI 10.1007/978-3-7091-1312-7_2,



1 (aflatoxin B_1) **2** (aflatoxin B_2)

3 (aflatoxin G_1) **4** (aflatoxin G_2)

Fig. 2.2 The aflatoxins B_1, B_2, G_1, and G_2 (**1–4**)

The most widely examined aflatoxin is aflatoxin B_1 (**1**), which is also the most toxic, carcinogenic, and mutagenic aflatoxin among all that are presently known (*17*, *18*). It was isolated together with aflatoxins B_2 (**2**), G_1 (**3**), and G_2 (**4**), which are shown in Fig. 2.2 (*19*). Their structures were revealed by the group of *Büchi* in 1963 (B_1 (**1**) and G_1 (**3**)) and 1965 (B_2 (**2**) and G_2 (**4**)) (*20*, *21*). This group also elucidated the absolute stereochemistry of aflatoxins in the B and G series by chemical degradation (*22*). Structurally, these compounds consist of five rings, having a furofuran moiety (rings B and C), an aromatic six-membered ring (A), a six-membered lactone ring (D), and either a five-membered pentanone or a six-membered lactone ring (E).

While the aflatoxins B and G are major compounds of the fungus *Aspergillus flavus*, there are also minor aflatoxin constituents from this organism, *e.g.* hydroxylated derivatives of aflatoxin B_1 (**1**) and B_2 (**2**), the so-called "milk-toxins", M_1 (**5**) and M_2 (**6**), which bear a hydroxy group at the junction of the two furan rings (*19*). They are called "milk toxins", because they are metabolites of aflatoxin B_1 (**1**) and B_2 (**2**), formed when cows get fed with contaminated foodstuffs. The toxins are then contained in the cow's milk. Other aflatoxins have a hydroxy group instead of

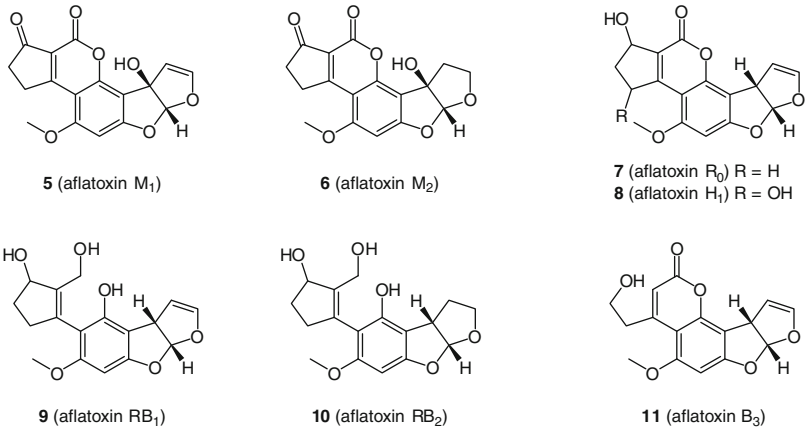
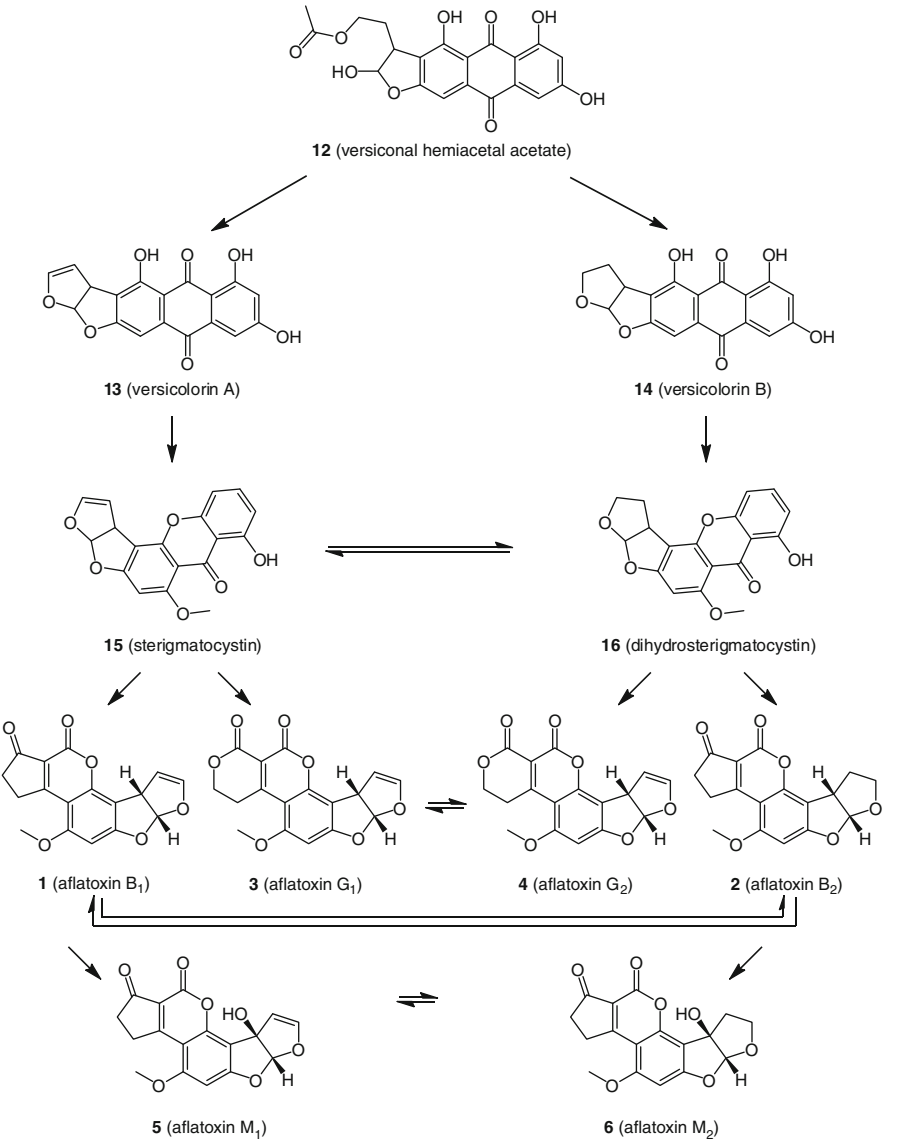


Fig. 2.3 Selected aflatoxins

a carbonyl group at ring E (R_0 (**7**), RB_1 (**9**), RB_2 (**10**), and H_1 (**8**)). They can be formed by microbial transformation or by chemical reduction with sodium borohydride (*23*, *24*). In some aflatoxins, the D-ring (RB_1 (**9**), RB_2 (**10**)) or the E-ring (B_3 (**11**)) is opened. Aflatoxin B_3 (**11**) is also called parasiticol, because it was first isolated from *Aspergillus parasiticus* (*23*). All aflatoxins shown in Fig. 2.3 are metabolic transformation products from the aflatoxins B (*19*).

Biosynthetically, the aflatoxins are all formed from the same precursor, versiconal hemiacetal acetate (**12**) (*25*). Compound **12** is formed from acetate, the units of which are converted into a polyketide. The polyketide is then metabolized to the xanthone **12** (see Scheme 2.1) (*26*). Intermediate **12** can then be transformed either into versicolorin A (**13**) or versicolorin B (**14**) in several steps. Versicolorin A (**13**) may be converted to sterigmatocystin (**15**), while **14** can lead to dihydrosterigmatocystin (**16**). Sterigmatocystin (**15**) can be metabolized to aflatoxins G_1 (**3**) or B_1 (**1**) and the latter may then be transformed to aflatoxin M_1 (**5**). Aflatoxins B_2 (**2**) and G_2 (**4**) are formed from dihydrosterigmatocystin (**16**) and aflatoxin M_2 (**6**) is formed by conversion from B_2 (**2**). Pathways also exist to convert aflatoxin B_1 (**1**) to B_2 (**2**), M_1 (**5**) to M_2 (**6**), and G_1 (**3**) to G_2 (**4**), and *vice versa*. Important biosynthesis steps are shown in Scheme 2.1.



Scheme 2.1 Biosynthesis of aflatoxins B (**1, 2**), G (**3, 4**), and M (**5, 6**); an arrow can represent more than one step

2.1 Biological Properties

Aflatoxins are acutely toxic compounds, and produce hepatic changes, which can cause serious liver damage (*27*). The liver is the main organ affected, followed by the kidneys. Hemorrhage, cirrhosis, and fatty degeneration of the liver are the most common effects on ingestion, but the pancreas, gall bladder, lung, and gut may also be affected (*28*).

When taken orally, the aflatoxins are absorbed from the gut and are transported to the liver where they are metabolized. For example, aflatoxin B_1 (**1**) may be transformed to aflatoxin M_1 (**5**), representing a detoxification, since aflatoxin M_1 (**5**) is less active than aflatoxin B_1 (**1**) (see below) (*27*). However, a common metabolic process is diol formation at the double bond of the furan ring. The resultant aflatoxin B_1-2,3-diol is much more toxic than aflatoxin B_1 (**1**) itself. Accordingly, diol formation results from metabolic activation to a very toxic species (*29*).

Among the naturally occurring aflatoxins, aflatoxin B_1 (**1**) is the most acutely toxic representative, followed by aflatoxins G_1 (**3**), B_2 (**2**), and G_2 (**4**). This is shown by LD_{50} values of one-day-old ducklings. While the LD_{50} of aflatoxin B_1 (**1**) is 0.36 mg/kg, the corresponding value for aflatoxin B_2 (**2**) is five times higher, with this compound containing a saturated furan ring. This shows that the unsaturated furan moiety has an important effect on acute toxicity. On comparing the LD_{50} value of aflatoxin G_1 (**3**) with that of B_1 (**1**), where the cyclopentanone ring has been converted in the former compound into a six-membered lactone ring, **3** is considerably less potent (0.78 mg/kg). Therefore, the cyclopentanone ring is of lesser importance for the mediation of acute toxicity (*27*, *30*).

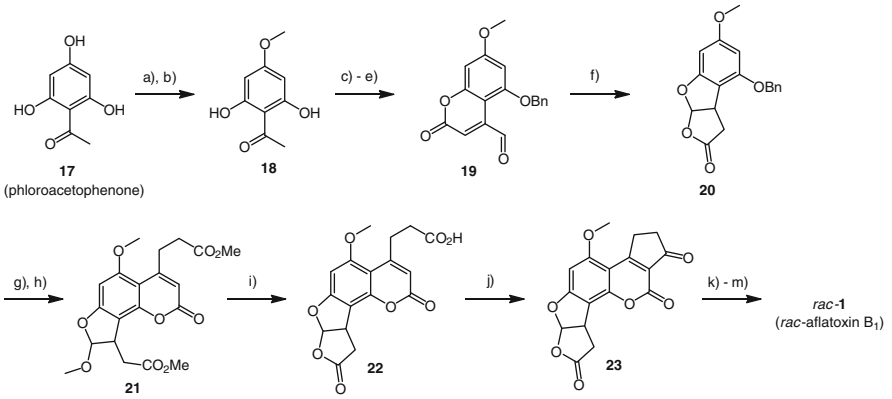
Besides their acute toxicity, aflatoxins are also highly carcinogenic. In fact, aflatoxin B_1 (**1**) is the most potent known liver carcinogen for mammals. It can not only induce tumors and metastases when directly injected, but also when it is given orally over a long period (*13*). Aflatoxins inhibit DNA-, RNA-, and protein biosynthesis by adduct formation (*14*, *31*, *32*). Their mutagenic potential is related to these biological effects. Structure-activity relationships for the carcinogenicity and mutagenicity of aflatoxins show the same general trends as for their acute toxicity. After aflatoxin B_1 (**1**), aflatoxin R_0 (**7**) is the most powerful mutagen, followed by aflatoxins M_1 (**5**), H_1 (**8**), B_2 (**2**), and G_2 (**4**) (*17*). When tested for their effects on chromosomes, aflatoxins cause a highly significant increase in the number of abnormal anaphases, with fragmentation of the chromosomes and inhibition of mitosis being observed (*13*).

The high toxicity and carcinogenicity of the aflatoxins makes it impractical to use them as pharmacological agents. Only very few studies have been carried out to investigate their potential as drugs or pesticides. In one study, it was shown that aflatoxins are able to inhibit sporulation of different fungi by inhibiting the activity of essential enzymes (*33*). However, the fact that they belong to the most toxic, carcinogenic, and mutagenic group of mycotoxins known, makes it improbable that these substances will ever be applied as therapeutic agents.

2.2 Total Syntheses of Aflatoxins

2.2.1 Total Syntheses of Racemic Aflatoxins

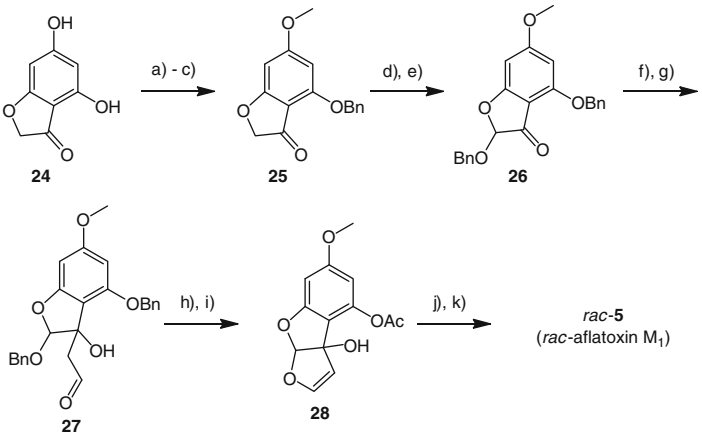
The group of *Büchi*, who also determined the structure and absolute configuration of several aflatoxins (*20–22*), achieved the first total synthesis of racemic aflatoxin B_1 (**1**) in 1966 (*34*, *35*). They started from phloroacetophenone (**17**), which was converted in two steps into its monomethyl ether **18** (see Scheme 2.2). Selective monobenzylation, followed by *Wittig* condensation and selenium dioxide oxidation gave the bicyclic aldehyde **19** in good yield.



Scheme 2.2 First total synthesis of aflatoxin B_1 (**1**), achieved by *Büchi et al.*. Reagents and conditions: a) Ac_2O, 110–165°C, 2 h, 40%; b) CH_2N_2, Et_2O/dioxane, rt; then HCl, MeOH, reflux, 8 h, 83%; c) BnBr, K_2CO_3, acetone, rt, 14 h, 82%; d) carbethoxymethylenetriphenylphosphorane, 170°C, 19 h, 72%; e) SeO_2, xylene, reflux, 5 h, 93%; f) Zn, HOAc, 100–120°C, 1.5 h, 80%; g) H_2, Pd/C, ethanol, rt, 2 h, quant; h) β-oxoadipate, HCl, MeOH, −12 to −20°C; then 3–5°C, 18 h, 57%; i) HOAc, H_2O, HCl (aq.), rt, 24 h, quant; j) $(COCl)_2$, CH_2Cl_2, 5°C to rt, 48 h; then $AlCl_3$, CH_2Cl_2, −5 to 5°C, 10 h; then HCl, rt, 2 h, 37%; k) disiamylborane, diglyme/THF, 60°C, 84 h, 16%; l) *p*-TsOH (cat.), Ac_2O, HOAc, rt, 12 h, 70%; m) 240°C, 15 min, 0.01 mm, 40%

Reduction of the double bond with zinc/glacial acetic acid and *in situ* rearrangement resulted in the tricyclic species **20**, which already possesses three of the five aflatoxin rings. Deprotection of the benzyl ether by hydrogenation, followed by a *Pechmann* condensation with ethyl methyl *β*-oxoadipate gave the lactone **21**. The two methyl esters and the methyl ether were hydrolyzed under acidic conditions and the lactone **22** formed immediately. Conversion of the acid into its chloride with oxalyl chloride formed the five-ring lactone **23**. Reduction to the corresponding lactol, acetoxylation, and pyrolysis gave racemic aflatoxin B_1 (**1**) in 13 steps and 0.9% overall yield from **17**.

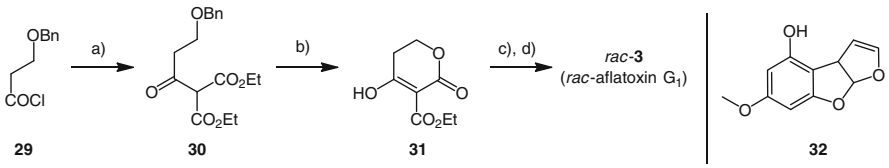
In 1969, *Büchi et al.* published the first total synthesis of racemic aflatoxin M_1 (**5**) (*36*). They started with the diol **24**, which was first dimethylated with dimethyl sulfate, then mono deprotected by aluminum chloride, and finally benzylated to afford species **25** (see Scheme 2.3).



Scheme 2.3 Total synthesis of racemic aflatoxin M_1 (**5**) by *Büchi et al.* Reagents and conditions: a) Me_2SO_4, K_2CO_3, dimethoxyethane, reflux, 3 h, 79%; b) $AlCl_3$, CH_2Cl_2, reflux, 1.25 h; then HCl, reflux, 64%; c) BnBr, K_2CO_3, dimethoxyethane/DMF, reflux, 74%; d) Me_3NPhBr_3, THF, 88%; e) $CaCO_3$, BnOH, Δ, 1.5 h, 65%; f) allylmagnesium bromide, THF/Et_2O, 0°C, 10 min; g) $NaIO_4$, OsO_4, $NaHCO_3$, dioxane/water, rt, 1 h, 63% over two steps; h) H_2, Pd/C, NaOAc, Ac_2O/benzene, rt, 1.5 h, 27%; i) toluene, 450°C, 73%; j) $NaHCO_3$, MeOH/H_2O, rt, 0.75 h, 94%; k) 2-carboxy-3-bromocyclopent-2-enone, $NaHCO_3$, $ZnCO_3$, CH_2Cl_2, rt, 20 h, 32%

Bromination at the α-position to the carbonyl group, and conversion into the benzyl ether gave acetal **26**. *Grignard* addition of allylmagnesium bromide to the ketone, followed by diol formation and oxidative glycol cleavage with sodium periodate and osmium tetroxide, yielded aldehyde **27**. Hydrogenolysis of the two benzyl ethers, followed by acetoxylation and pyrolysis gave the tricyclic alcohol **28**. The acetoxy group was cleaved by basic hydrolysis and the resulting alcohol was coupled with 2-carboxyethyl-3-bromocyclopent-2-enone to give racemic aflatoxin M_1 (**5**) in 11 linear steps from **24** and 0.7% overall yield.

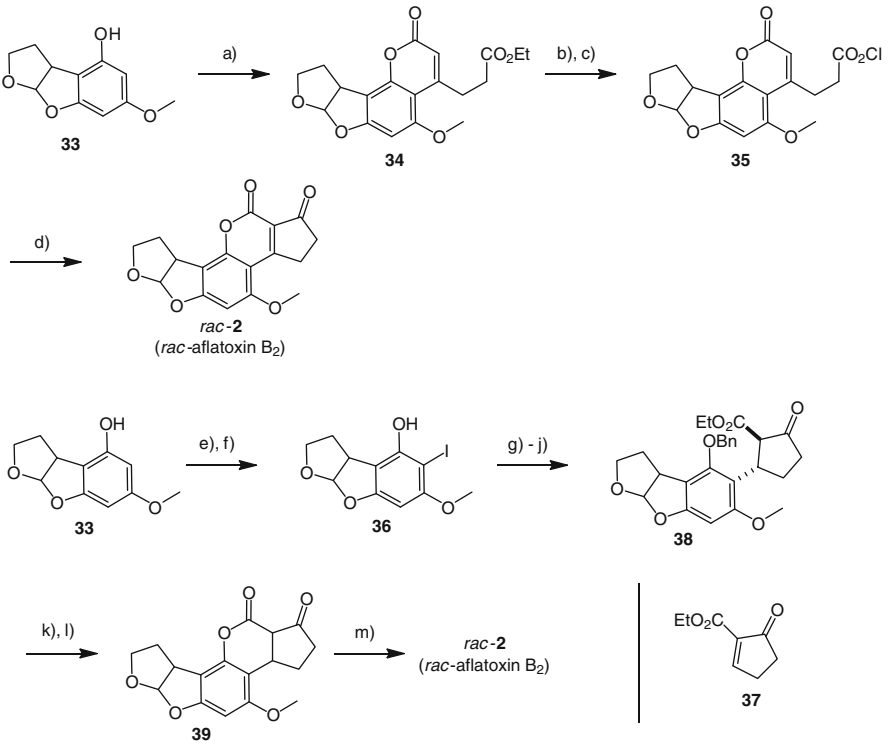
One year later, in 1970, *Büchi* and *Weinreb* presented a total synthesis of racemic aflatoxin G_1 (**3**) and an improved synthesis of aflatoxin B_1 (**1**) (*37*). The synthesis of **1** involved the same coupling with a cyclopentenone as described above for the total synthesis of aflatoxin M_1 (**5**) (see last step in Scheme 2.3). Accordingly, this group was able to increase the overall yield to 2.5% with the same number of reaction steps.



Scheme 2.4 Total synthesis of racemic aflatoxin G_1 (**3**). Reagents and conditions: a) diethylmalonate, Mg, ethanol/CCl_4, 0°C; then Et_2O, reflux, 3 h; then **29**, Et_2O, rt, 2 h, 97%; b) H_2, Pd/C, EtOAc, rt, 2 h, 64%; c) $(COBr)_2$, benzene, rt, 96%; d) 32, $ZnCO_3$, LiI, CH_2Cl_2, rt, 3 h; then reflux, 7 h; then rt, 14%

The synthesis of aflatoxin G_1 (**3**) is shown in Scheme 2.4. The acid chloride **29** was coupled with diethyl malonate (→ **30**), then the benzyl protecting group was removed by hydrogenolysis and lactone **31** formed. Conversion of the hydroxy group into the bromide with oxalyl bromide, followed by coupling with building block **32** gave racemic aflatoxin G_1 (**3**). Different syntheses of the tricycle **32** are presented in Sect. 2.3.2.

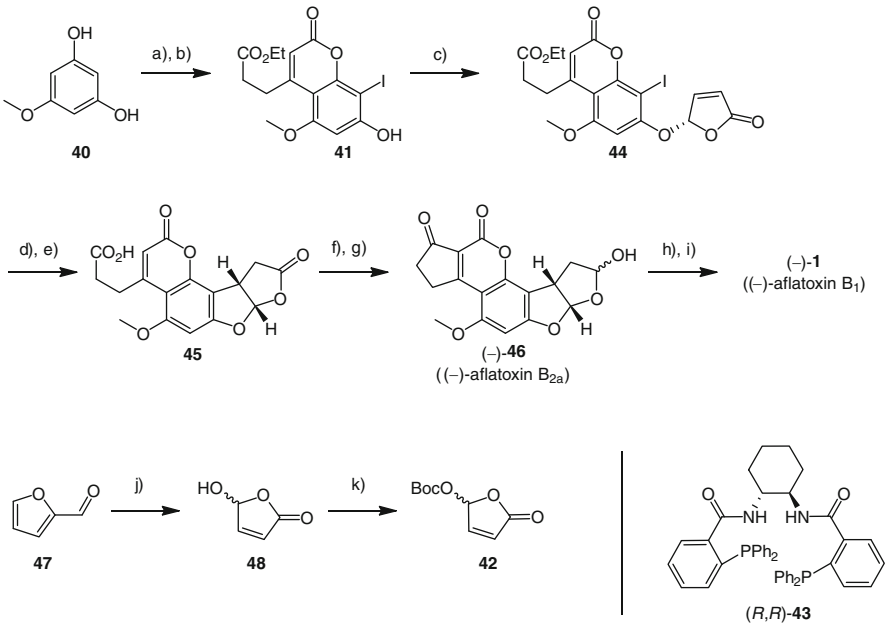
Aflatoxin B_2 (**2**) was first synthesized by *Roberts et al*. in 1968 (*38*). They started from the tricyclic compound **33**, for which the synthesis is described in Sect. 2.3.1. *Pechmann* condensation with diethyl β-oxoadipate generated the lactone **34**. Hydrolysis of the ethyl ester, followed by acid chloride formation with oxalyl chloride, gave **35**. This was used without further purification for a *Friedel-Crafts* acylation reaction to yield racemic aflatoxin B_2 (**2**). The synthesis is presented in Scheme 2.5, which also shows another total synthesis of aflatoxin B_2 (**2**). The second one was published in 1990 by *Horne et al*. (*39*). This group started from the same intermediate **33** and first diiodinated it. Regioselective deiodination gave **36**. The free alcohol was then protected as a benzyl ether, then a metal halogen exchange was realized with *n*-BuLi, followed by a transmetalation with lithium 2-thienylcyano cuprate. Final cuprate addition to the cyclopentanone **37** gave **38**. Cleavage of the benzyl ether by hydrogenolysis and acidic cleavage of the ester group produced the five-ring-species **39** *in situ*. Oxidation to aflatoxin B_2 (**2**) was achieved with DDQ.



Scheme 2.5 Syntheses of aflatoxin B_2 (**2**) by *Roberts et al.* (above) and by *Horne et al.* (below). Reagents and conditions: a) diethyl β-oxoadipate, HCl, ethanol, rt, 19%; b) KOH, ethanol, reflux, 2 h, 76%; c) $(COCl)_2$, CH_2Cl_2; d) $AlCl_3$, CH_2Cl_2, −5°C, 3 h, 38% over two steps; e) $Me_3BnNICl_2$, $MeOH/CH_2Cl_2$; f) NaH, 0°C; then *n*-BuLi, −100°C, 15 min, 70%; g) BnBr, K_2CO_3; h) *n*-BuLi, −78°C; i) lithium 2-thienylcyano cuprate, −78°C to 0°C; j) **37**, −78°C to rt, 60% over three steps; k) H_2, Pd/C, EtOAc, rt, 9 h, 200 psi; l) TFA, CH_2Cl_2, rt, 60% over two steps; m) DDQ, dioxane, rt, quant

2.2.2 *Enantioselective Total Syntheses of Aflatoxins*

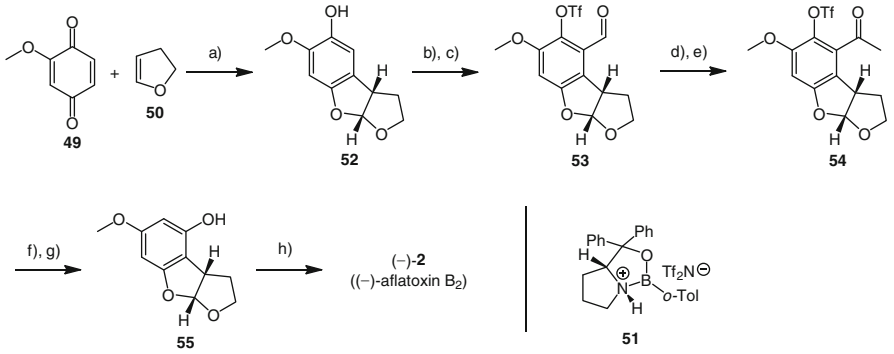
In 2003, *Trost* and *Toste* presented the first enantioselective total synthesis of aflatoxins B_1 (**1**) and B_{2a} (**46**) (*40*, *41*). In Scheme 2.6, their synthesis is shown. The starting material for this sequence is catechol **40**. A *Pechmann* condensation with diethyl β-oxoadipate and iodination with iodine chloride gave the lactone **41**.



Scheme 2.6 Enantioselective total synthesis of (–)-aflatoxin B_{2a} (**46**) and (–)-aflatoxin B_1 (**1**). Reagents and conditions: a) diethyl β-oxoadipate, HCl, ethanol, rt, 3 d, 47%; b) ICl, CH_2Cl_2, rt, 30 min, 92%; c) **42**, Pd_2dba_3•$CHCl_3$, (*R*,*R*)-**43**, tetrabutylammonium chloride, CH_2Cl_2, rt, 12 h, 89%; d) $(CH_3CN)_2PdCl_2$, NEt_3, DMF, 60°C, 1 h, 93%; e) HCl, HOAc, H_2O, rt, 2 d, quant; f) Sc $(OTf)_3$, $LiClO_4$, CH_3NO_2, 60°C, 4 h, 32%; g) DIBAL-H, CH_2Cl_2, −78°C, 1 h, 57%; h) Ac_2O, HOAc, rt, 20 h; i) 240°C, 15 min, 24% over two steps; j) Rose Bengal, O_2, MeOH, 450 W Hg lamp, 8 h; k) Boc_2O, pyridine, THF, rt, 12 h, 61% over two steps

The stereogenic centers were then introduced by palladium-catalyzed dynamic kinetic asymmetric transformation. Therefore, **41** was coupled with lactone **42** in the presence of chiral ligand (*R*,*R*)-**43** and gave **44** in 89% yield. The synthesis of **42** is shown below in Scheme 2.6. Compound **44** was subjected to an intramolecular *Heck* reaction followed by acidic cleavage of the ester function (→ **45**). The intramolecular *Heck* reaction only produced one diastereomer, because the *cis*-annelated rings are favored. Scandium(III)-mediated cyclization and reduction of the lactone with DIBAL-H yielded (–)-aflatoxin B_{2a} (**46**). It was acetoxylated and then pyrolyzed to give (–)-aflatoxin B_1 (**1**) in 1.6% overall yield and nine linear steps from catechol (**40**).

In 2005, *Zhou* and *Corey* presented an enantioselective total synthesis of aflatoxin B_2 (**2**) (*42*). This is shown in Scheme 2.7. The stereospecificity was induced in the first step by an asymmetric [3 + 2]-cycloaddition with a chiral borazine. Methoxy *p*-benzoquinone (**49**) reacted with dihydrofuran (**50**) in the presence of **51** and gave **52** in 99% enantiomeric excess. Sequential *ortho*-formylation and triflate ester formation yielded **53**. Ketone **54** was formed by *Grignard* reaction and *Dess-Martin*-periodinane oxidation. *Baeyer-Villiger* oxidation and reductive removal of the triflate group, together with deacetoxylation produced the alcohol **55**. Conversion into (–)-aflatoxin B_2 ((–)-**2**) (2.5% overall yield for eight steps) was achieved by coupling with 3-bromo-2-carboxyethyl-cyclopent-2-enone.

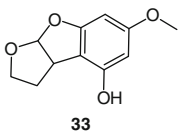


Scheme 2.7 Enantioselective total synthesis of aflatoxin B_2 (**2**). Reagents and conditions: a) **51**, CH_2Cl_2/CH_3CN, −78°C to rt, 7 h, 65%, 99% *ee*; b) hexamethylenetetramine, HOAc, 110°C, 48 h, 40%; c) DMAP (cat.), pyridine, Tf_2O, CH_2Cl_2, −20°C to 0°C, 80%; d) MeMgBr, THF, −20°C, 2 h; e) *DM*P, CH_2Cl_2, 0°C to rt, 85% over two steps; f) TFAA, urea•H_2O, CH_2Cl_2, rt, 63%; g) Raney-Ni, H_2, MeOH, rt, 3 h, 60%; h) $NaHCO_3$, $ZnCO_3$, ethyl 2-bromo-5-oxocyclopent-1-enecarboxylate, CH_2Cl_2, rt, 20 h, 36%

2.3 Syntheses of Aflatoxin Building Blocks

2.3.1 Syntheses of Building Blocks for Aflatoxins B_2 and G_2

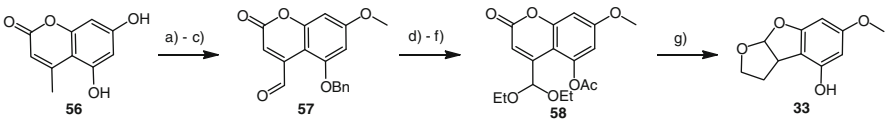
There are many different syntheses for the important building block **33** (Fig. 2.4). From this molecule, one can easily build aflatoxins B_2 (**2**) and G_2 (**4**) by the reactions presented in Sect. 2.2.



33

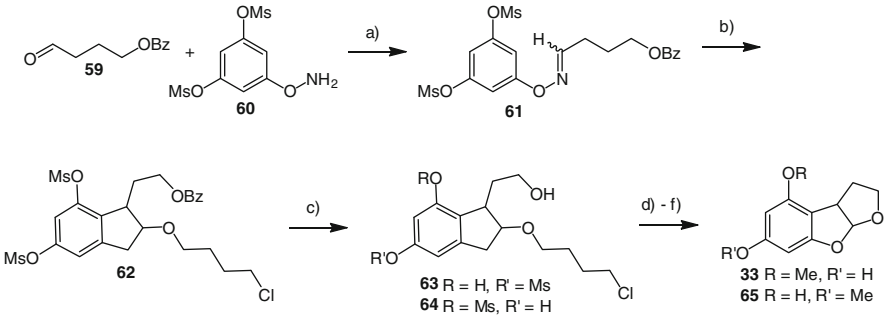
Fig. 2.4 Building block **33** for aflatoxins B_2 (**2**) and G_2 (**4**)

The first access to **33** was published by *Knight et al*. in 1966 and is presented in Scheme 2.8 (*43*). The diol **56** was monomethylated, benzylated, and then oxidized by selenium dioxide (→ **57**). The acetal was then formed with ethanol, the benzyl group was removed with hydrogen, and the resulting alcohol was converted into acetate **58**. Reduction of the lactone to the lactol afforded ring opening and following acidic hydrolysis of the acetate gave the desired building block **33** in 5.3% overall yield.



Scheme 2.8 First synthesis of **33**. Reagents and conditions: a) Me_2SO_4, Na_2CO_3, H_2O, 80°C, 0.5 h, 33%; b) BnCl, NaI, Na_2CO_3, acetone, reflux, 8 h, 81%; c) SeO_2, xylene, reflux, 6 h, 59%; d) HCl, EtOH, $(EtO)_3CH$, rt to 50°C; then rt, 89%; e) H_2, *Adams* catalyst, EtOAc, rt, 88%; f) Ac_2O, pyridine, 86%; g) $LiAlH_4$, Et_2O, reflux, 4 h; then HCl, 50%

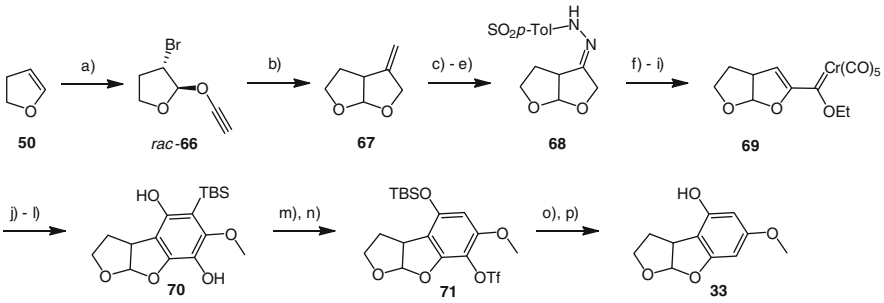
A straightforward access to **33** in six steps and 49% overall yield was published by *Castellino* and *Rapoport* in 1985 and is shown in Scheme 2.9 (*44*). The first step was an imine formation (→ **61**). By heating under acidic conditions, an oxaza-*Cope* rearrangement occurred, which, after hydrolysis, led to ring closure to the furan **62**. Under these conditions, the benzoyl group was cleaved. The free alcohol was then protected by degradation products of the solvent THF, which were formed by acid cleavage. Basic hydrogenolysis gave the regioisomers **63** and **64**, which were not separated. With catalytic amounts of *p*-TsOH under heating, ring closure occurred. The free alcohol was then methylated and the mesyl group was removed to form **33** together with its regioisomer **65**.



Scheme 2.9 Short access to **33** *via* oxaza-*Cope* rearrangement. Reagents and conditions: a) HCl, ethanol, reflux, 83%; b) HCl, THF, 65°C, 24 h, 87%; c) LiOH•H_2O, THF/H_2O, 40°C, 1 d, 95%; d) *p*-TsOH (cat.), 4 Å activated sieves, CH_3CN, rt, 45 min, 95%; e) Me_2SO_4, K_2CO_3, CH_3CN, rt, 1.75 h, 93%; f) Et_4NOH, THF/H_2O, reflux, 5 h, quant

Other syntheses of **33** have been presented in more recent years: *Weeratunga et al.* presented a nine-step-synthesis with 4% overall yield (*45*), where the key steps were a cyclization-deiodination-reaction and a lead tetraacetate-conducted ring closure. *Koreeda et al.* published their building-block-synthesis in 1993 with 11% overall yield (*46*), and in 1996, *Pirrung* and *Lee* synthesized **33** *via* a rhodium carbenoid dipolar cycloaddition (*47*).

A recent synthesis of this building block has been published by *Eastham et al.* in 2006 (*48*). Their key step is a *Dötz* benzannulation reaction and is shown in Scheme 2.10. The bromohydrin **66** was formed from dihydrofuran (**50**). Cobalt-mediated cyclization, followed by ozonolysis with reductive work-up yielded **68** after hydrazine formation. Reductive removal of the hydrazine function, followed by chromium-carbonyl formation gave the *Dötz* reaction precursor **69**. This reacted with an alkyne in the *Dötz* reaction, and was then oxidized and hydrogenated (→ **70**). Pyrolysis gave the protected alcohol and the remaining free alcohol was protected as a triflate (→ **71**). Reductive removal of the triflate and deprotection of the silyl ether yielded the desired **33** in 1.2% overall yield.



Scheme 2.10 Synthesis of **33** *via* a *Dötz* reaction. Reagents and conditions: a) prop-2-yn-1-ol, NBS, CH_2Cl_2, 94%; b) CoL_n, $NaBH_4$, NaOH, ethanol, 62%; c) O_3, CH_2Cl_2; d) Me_2S, 74% over two steps; e) *p*-$TolSO_2NHNH_2$, THF, 79%; f) Na, triglycol, 120°C, 73%; g) *t*-BuLi, THF, −78°C; h) $Cr(CO)_6$; i) Et_3OBF_4, 52% over three steps; j) *t*-butyl(methoxyethynyl)dimethylsilane, THF, 80°C, 31%; k) CAN, H_2O/CH_3CN, 0°C, 10 min, 93%; l) H_2, Pd/C, EtOAc, quant; m) toluene, 110°C, quant; n) Tf_2O, pyridine, DMAP (cat.), CH_2Cl_2, 93%; o) Raney-Ni, MeOH; p) TBAF, THF, 35% over two steps

2.3.2 *Syntheses of Building Blocks for Aflatoxins B_1 and G_1*

There exist many references describing the syntheses of aflatoxin B_1 and G_1 building blocks. Since aflatoxin B_1 (**1**) can be converted *via* hydrogenolysis into aflatoxins B_2 (**2**) and G_1 (**3**) into G_2 (**4**), the building blocks described in this chapter can also be precursors for aflatoxins B_2 (**2**) and G_2 (**4**).

There are different syntheses for unsubstituted model systems of aflatoxin precursors. However, these cannot be used for total synthesis (Fig. 2.5). Compound **72** has been synthesized by *Pawlowski et al.* in four steps (*49*). Compound **73** was obtained in four steps by *Snider et al. via* a ketene-[2 + 2]-cycloaddition and a *Baeyer-Villiger* oxidation (*50*). *Mittra et al.* synthesized **74** in the same way as *Snider et al.* (*51*).