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The Chemistry of Mycotoxins

Bearbeitet von
Stefan Bräse, Franziska Gläser, Carsten Kramer, Stephanie Lindner, Anna M. Linsenmeier, Kye-Simeon Masters, Anne C. Meister, Bettina M. Ruff, Sabilla Zhong

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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*).

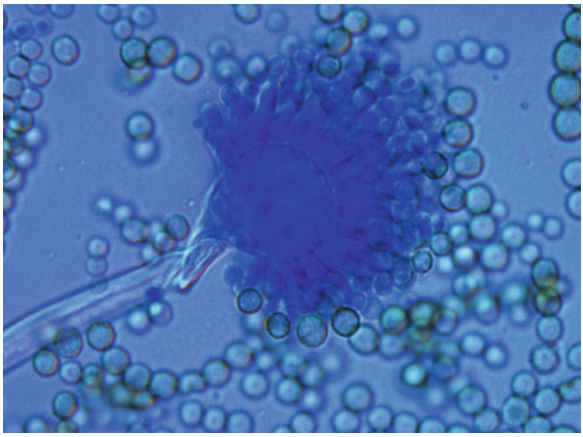

Fig. 2.1 *Aspergillus flavus* spores as seen under the light microscope under 600-fold magnification

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*).

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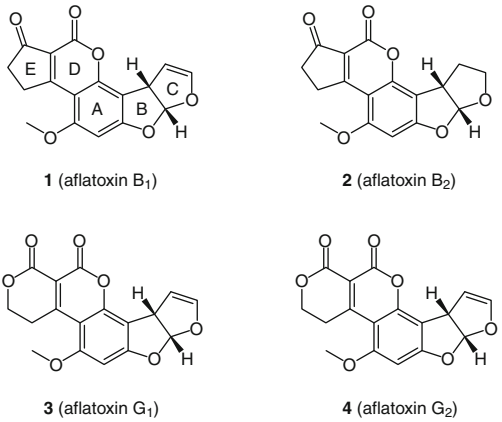


1 (aflatoxin B_1) **2** (aflatoxin B_2)

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

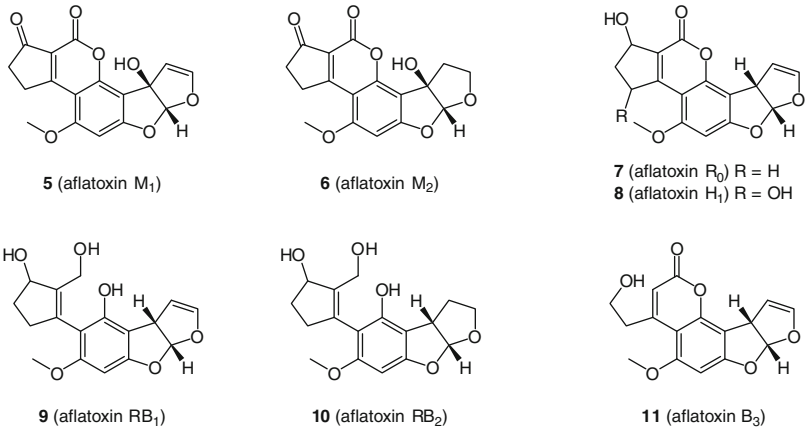
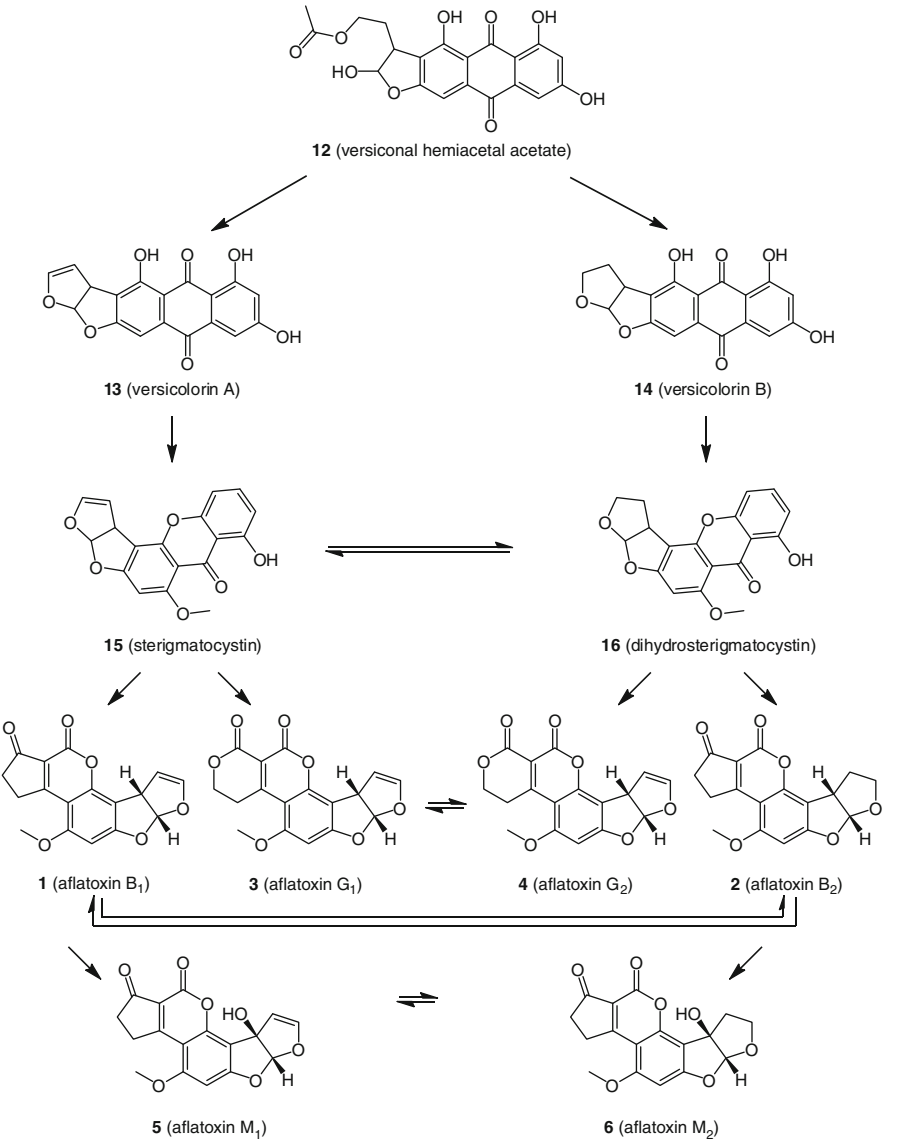


Fig. 2.3 Selected aflatoxins

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*).

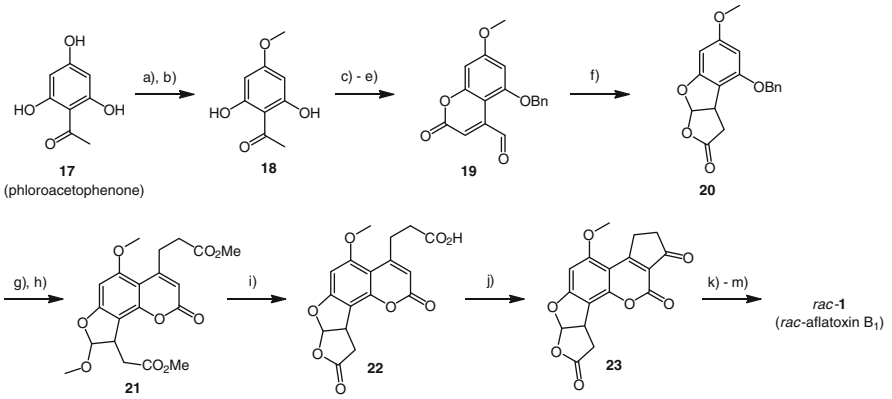
Besides their acute toxicity, aflatoxins are also highly carcinogenic. In fact, aflatoxin B_1 (**1**) is the most potent known liver carcinogen for mammals. It can not only induce tumors and metastases when directly injected, but also when it is given orally over a long period (*13*). Aflatoxins inhibit 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

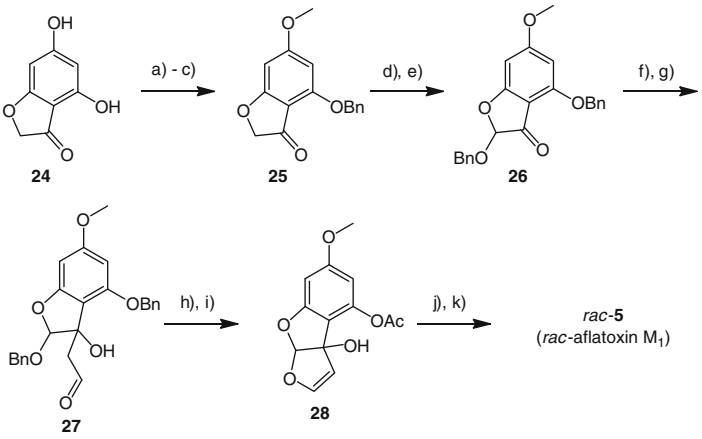
The group of *Büchi*, who also determined the structure and absolute configuration of several aflatoxins (*20–22*), achieved the first total synthesis of racemic aflatoxin B_1 (**1**) in 1966 (*34*, *35*). They started from phloroacetophenone (**17**), which was converted in two steps into its monomethyl 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**.

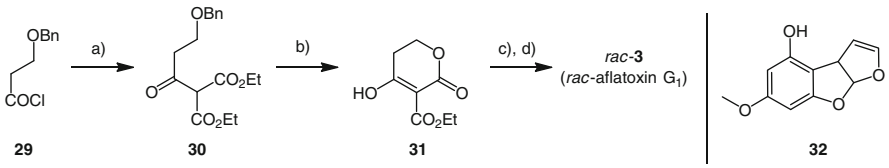
In 1969, *Büchi et al.* published the first total synthesis of racemic aflatoxin M_1 (**5**) (*36*). They started with the diol **24**, which was first dimethylated with dimethyl sulfate, then mono deprotected by aluminum chloride, and finally benzylated to afford species **25** (see Scheme 2.3).



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.

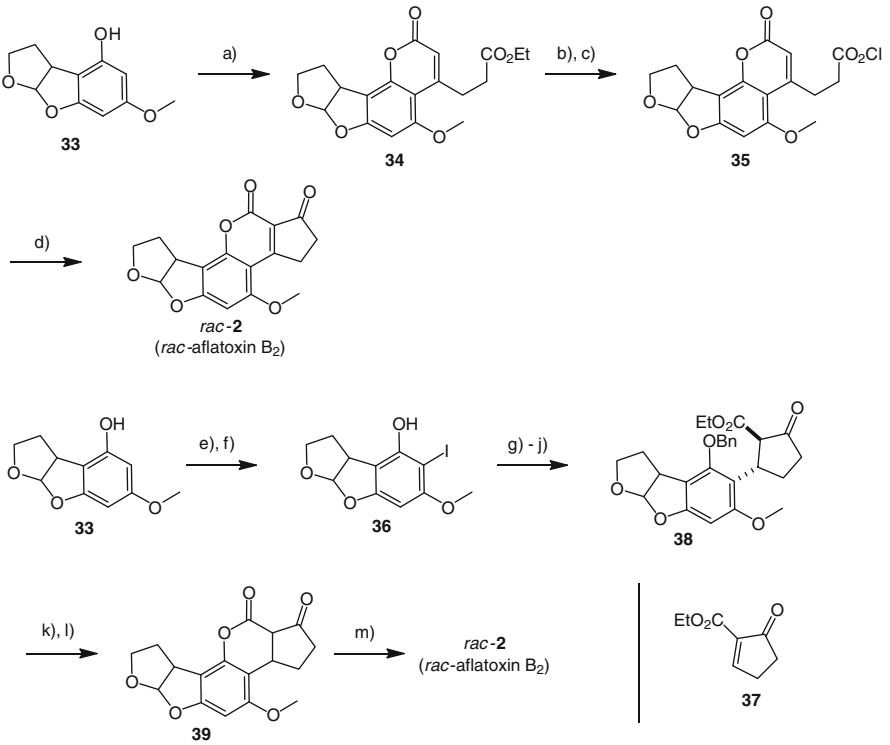
One year later, in 1970, *Büchi* and *Weinreb* presented a total synthesis of racemic aflatoxin G_1 (**3**) and an improved synthesis of aflatoxin B_1 (**1**) (*37*). The synthesis of **1** involved the same coupling with a cyclopentenone as described above for the total synthesis of aflatoxin M_1 (**5**) (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.

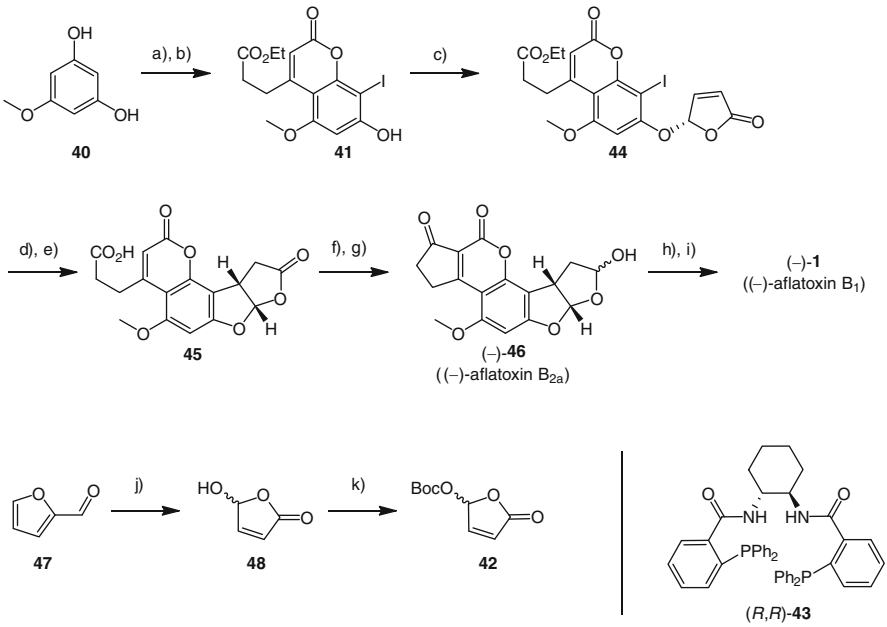
Aflatoxin B_2 (**2**) was first synthesized by *Roberts et al*. in 1968 (*38*). They started from the tricyclic compound **33**, for which the synthesis is described in Sect. 2.3.1. *Pechmann* condensation with diethyl β-oxoadipate generated the lactone **34**. Hydrolysis of the ethyl ester, followed 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*

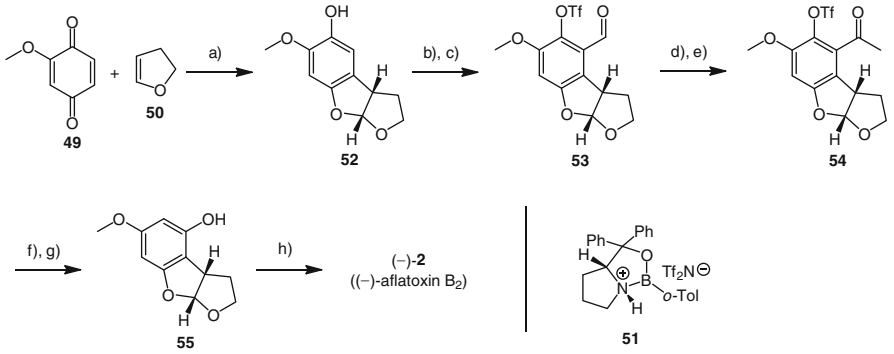
In 2003, *Trost* and *Toste* presented the first enantioselective total synthesis of aflatoxins B_1 (**1**) and B_{2a} (**46**) (*40*, *41*). In Scheme 2.6, their synthesis is shown. The starting material for this sequence is catechol **40**. A *Pechmann* condensation with diethyl β-oxoadipate and iodination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

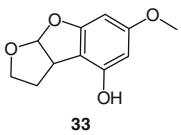


Scheme 2.7 Enantioselective total synthesis of aflatoxin B_2 (**2**). Reagents and conditions: a) **51**, CH_2Cl_2/CH_3CN, −78°C to rt, 7 h, 65%, 99% *ee*; b) hexamethylenetetramine, HOAc, 110°C, 48 h, 40%; c) DMAP (cat.), pyridine, Tf_2O, CH_2Cl_2, −20°C to 0°C, 80%; d) MeMgBr, THF, −20°C, 2 h; e) *DM*P, 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

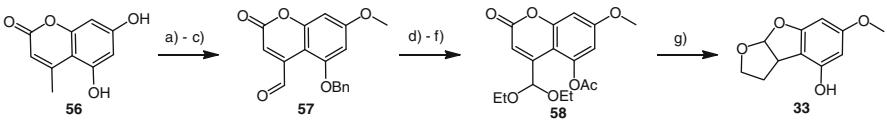
There are many different syntheses for the important building block **33** (Fig. 2.4). From this molecule, one can easily build aflatoxins B_2 (**2**) and G_2 (**4**) by the reactions presented in Sect. 2.2.



33

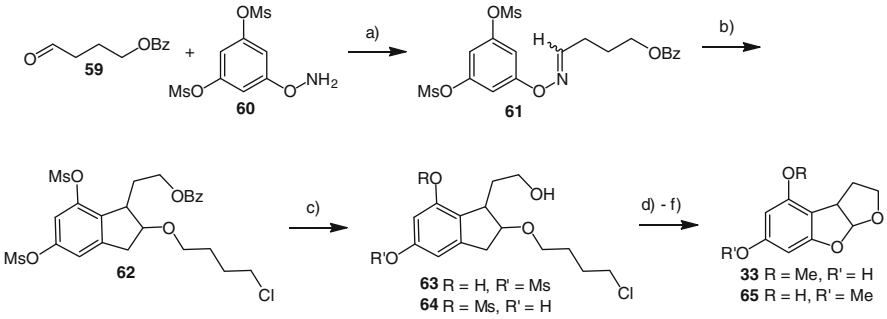
Fig. 2.4 Building block **33** for aflatoxins B_2 (**2**) and G_2 (**4**)

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%

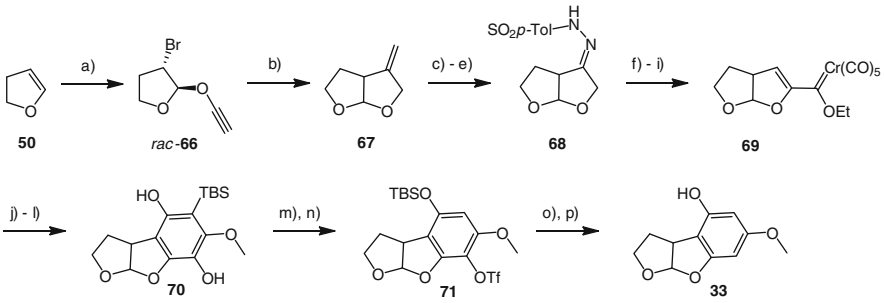
A straightforward access to **33** in six steps and 49% overall yield was published by *Castellino* and *Rapoport* in 1985 and is shown in Scheme 2.9 (*44*). The first step was an imine formation (→ **61**). By heating under acidic conditions, an oxaza-*Cope* rearrangement occurred, which, after hydrolysis, 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*).

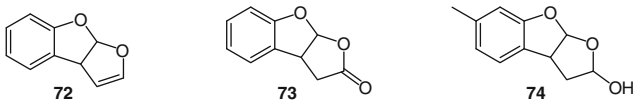
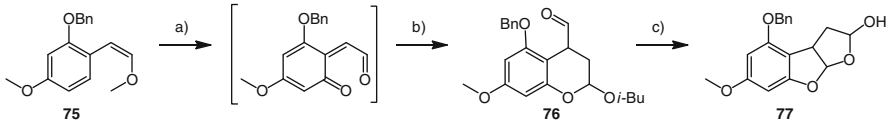


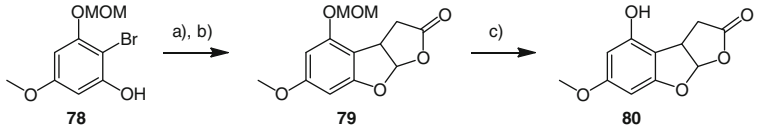
Fig. 2.5 Model systems for aflatoxin precursors

Matsumoto and *Kuroda* presented a short and elegant synthesis for an aflatoxin B_1 precursor by a [2 + 4]-cycloaddition with singlet oxygen (see Scheme 2.11). From **75**, an intermediate was formed that reacted with *iso*-butyl vinyl ether (→ **76**). Acid hydrolysis gave the free alcohol, which induced the formation of **77** (*52*).



Scheme 2.11 *Matsumoto*'s synthesis of **77**. Reagents and conditions: a) $^1O_2$; b) *i*-butyl vinyl ether, 39%; c) H_2SO_4 (cat.)

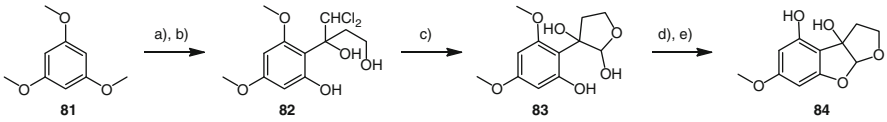
In 1988, *Sloan et al*. presented a building-block synthesis *via* radical-induced ring closure (*53*). The aromatic alcohol **78** was first substituted on 5-bromofuran-2(5*H*)-one, then an intramolecular, radical 1,4-addition formed **79**. Removal of the MOM-protecting group then gave **80**. The synthesis is shown in Scheme 2.12. From this intermediate, *Büchi et al*. described the synthesis of aflatoxin B_1 (**1**) (*35*). Other syntheses of building block **80** have been described by *Hoffmann et al*. and *Bujons et al*. (*54*, *55*).



Scheme 2.12 Building block synthesis *via* radical ring closure. Reagents and conditions: a) 5-bromofuran-2(5*H*)-one, K_2CO_3, acetone, reflux; b) Bu_3SnH, AIBN, benzene, reflux; c) 9-BBN-Br, CH_2Cl_2, −78°C to 0°C, 1.5 h

2.3.3 Synthesis of a Building Block for Aflatoxin M_2

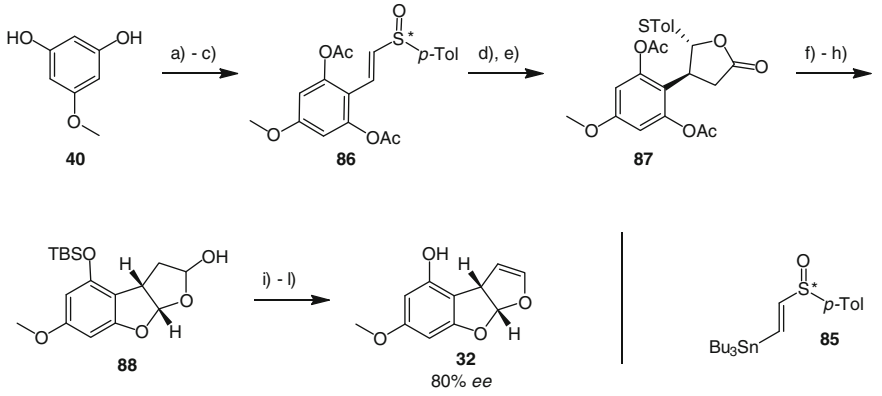
For aflatoxin M_2 (**6**), the required building block has been synthesized by *Kraus* and *Wang*, as shown in Scheme 2.13 (*56*). The starting material, 1,3,5-trimethoxybenzene (**81**), was first acylated and mono-demethylated *in situ*, then a 1,2-addition to the ketone provided **82**. Under basic conditions, ring closure and hydrolysis of the remaining chloride occurred and gave hemiacetal **83**. With *p*-toluenesulfonic acid, the last ring was closed, and with boron trifluoride, selective mono-demethylation yielded the desired building block **84**. Conversion into aflatoxin M_2 (**6**) can be achieved according to the protocol of *Büchi* for the synthesis of aflatoxin M_1 (**5**) (*36*, *37*).



Scheme 2.13 Synthesis of building block **84** for aflatoxin M_2 (**6**). Reagents and conditions: a) $AlCl_3$, oxetan-2-one, 80–85%; b) $LiCHCl_2$, THF, 93%; c) K_2CO_3, *i*-PrOH (aq.), 70%; d) *p*-TsOH, CH_2Cl_2, 4 h, 74%; e) BF_3•OEt_2, NaI, 71%

2.3.4 Enantioselective Syntheses of Aflatoxin Building Blocks

The first enantioselective synthesis of an aflatoxin building block was published in 1993 by *Marino* (*57*). He presented a synthesis of **32** in 80% enantiomeric excess and induced the stereospecificity *via* optically active vinyl sulfoxides (see Scheme 2.14). Catechol (**40**) was acylated, mono-iodinated and then coupled with chiral vinyl sulfoxide **85** under *Stille* conditions (→ **86**). Dichloroketene lactonization under reductive conditions followed by zinc-promoted dechlorination gave the major diastereomer **87**.

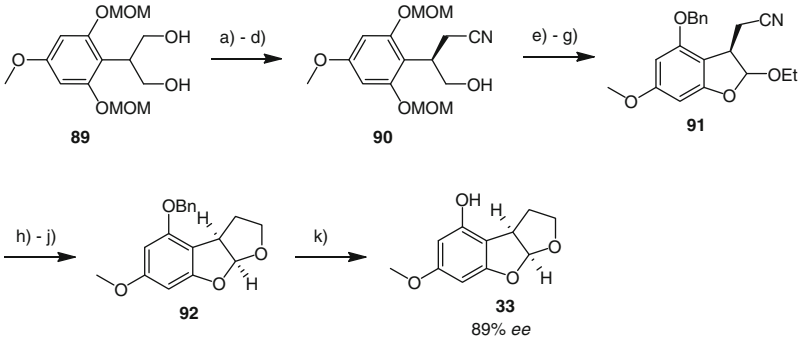


Scheme 2.14 Enantioselective synthesis of a building block (**28**) for aflatoxin B_1 (**1**). Reagents and conditions: a) AcCl, pyridine, 98%; b) HgO•HBF_4•SiO_2, I_2, 49%; c) 85, Pd(0), PPh_3, toluene, reflux, 65%; d) Zn(Cu), Cl_3CCOCl, THF, −50°C; e) Zn, HOAc, Δ, 70% over two steps, quant *ee*; f) HCl, acetone, Δ, 55%; g) TBSCl, imidazole; h) DIBAL-H, 80% over two steps; i) 1-(phenylthio) pyrrolidine-2,5-dione, PBu_3, benzene, 80%; j) *m*-CPBA, CH_2Cl_2, −78°C; k) pyridine, toluene, 110°C; l) CsF, CH_3CN, 0°C, 96% over three steps

With HCl, deacetylation and ring closure occurred. Then, the free aromatic alcohol was TBS-protected and the lactone was reduced with DIBAL-H to lactol **88**. The alcohol was converted into the thio ether, then oxidized with *m*-CPBA, and finally pyrolyzed. Fluoride-driven deprotection of the TBS ether then gave building block **32** in 80% *ee*.

In 1994, *Civitello* and *Rapoport* presented a further enantioselective synthesis of an aflatoxin B_1 building block with an oxaza-*Cope* rearrangement as a key step (*58*).

For aflatoxin B_2 (**2**), enantioselective syntheses of precursors have also been reported. *Shishido* and *Bando* presented their procedure in 1997, which gave an *ee* of 89% (*59*, *60*). The stereospecificity was induced by lipase-catalyzed monoacetoxylation of diol **89** (see Scheme 2.15). The remaining alcohol was mesyl-protected, converted into its cyanide and then deacetoxylated (→ **90**). With TPAP/NMO, the alcohol was oxidized to the aldehyde, then the MOM-groups were removed under acidic conditions, which caused lactolization. With triethoxyethane, the alcohol was protected *in situ*, and, in the next step, the remaining aromatic alcohol was benzylated (→ **91**). Under basic conditions, the nitrile was converted into the corresponding carboxylate, which was reduced to the alcohol by borane. With *p*-TsOH, ring closure afforded **92**. Hydrogenolysis of the benzyl group gave building block **33**, which can be converted into aflatoxin B_2 (**2**) according to *Büchi*'s or *Robert*'s conditions (*37*, *38*).

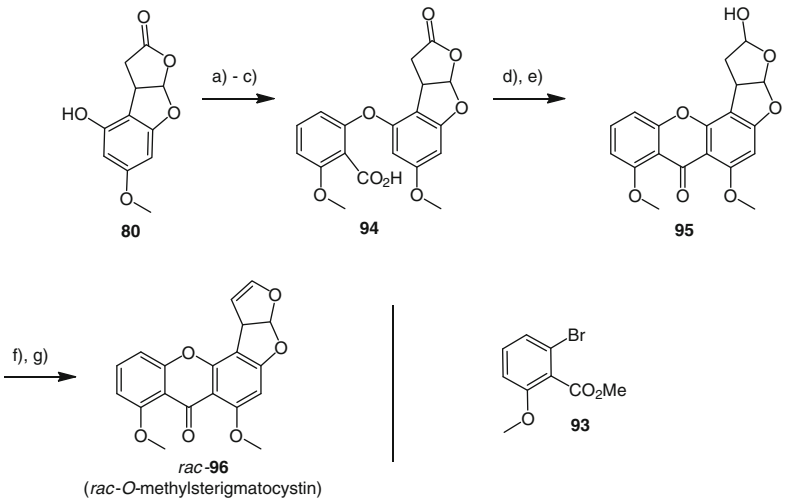


Scheme 2.15 Enantioselective synthesis of the aflatoxin B_2 building block **33**. Reagents and conditions: a) Lipase AL, vinyl acetate, Et_2O, rt, 72%, 89% *ee*; b) MsCl, DIPEA, DMAP, CH_2Cl_2, 89%; c) KCN, 18-Crown-6, DMSO, 72%; d) LiOH, THF/H_2O, 83%; e) TPAP, NMO, 4 Å MS, CH_2Cl_2; f) HCl, $HC(OEt)_3$, EtOH; g) BnCl, K_2CO_3, DMF, 50% over three steps; h) KOH, EtOH/H_2O; i) BH_3•SMe_2, THF; j) *p*-TsOH, CH_2Cl_2, 43% over three steps; k) 1,4-cyclohexandiene, Pd/C, MeOH, quant

2.4 Syntheses of Biosynthetic Aflatoxin Precursors

Various biosynthetic precursors of aflatoxins have been synthesized. Some of these have then been converted biosynthetically into the aflatoxins. In this section, syntheses of important aflatoxin precursors will be presented.

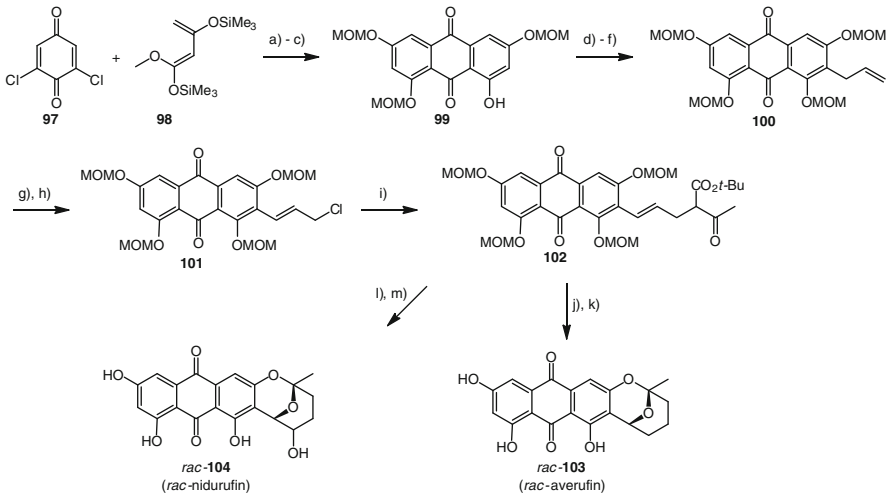
In 1971, *rac-O*-methylsterigmatocystin (OMST, **96**) was synthesized by *Rance* and *Roberts* (*61*). With respect to biosynthesis, this is an important intermediate between sterigmatocystin (**15**) and the aflatoxins B_1 (**1**) and G_1 (**3**) (see Scheme 2.1). The synthesis starts with building block **80** (for its synthesis see Scheme 2.12), which was ring-opened and methyl-protected under acidic conditions (Scheme 2.16). *Ullmann* coupling with bromide **93**, followed by acidic ester and ether hydrolysis led to ring closure and gave **94**. The carboxylic acid was converted into its chloride with oxalyl chloride, which reacted *in situ* to a xanthone species. Reduction of the lactone with disiamylborane gave lactol **95**. The alcohol was acylated and *rac-O*-methylsterigmatocystin (**96**) was obtained by repeated sublimation.



Scheme 2.16 Synthesis of *rac-O*-methylsterigmatocystin (**96**). Reagents and conditions: a) HCl, MeOH, −10°C, 1 h; then rt, 18 h, 75%; b) NaOMe, MeOH; then pyridine, **93**, CuCl, reflux, 4 h, 41%; c) HOAc, HCl, rt, 88%; d) $(COCl)_2$, benzene, reflux, 24 h, 71%; e) disiamylborane, THF, reflux, 48 h, 17%; f) HOAc, Ac_2O, *p*-TsOH (cat.), rt, 7 d, 49%; g) 250°C, 0.05 mm, 53%

Another synthesis of *rac-O*-methylsterigmatocystin (**96**) was published by *Casillas* and *Townsend* in 1999 (*62*). They used *N*-alkylnitrilium salts and a carbonyl-alkene interconversion as key steps for synthesizing *O*-methylsterigmatocystin (**96**) in 19 steps (see Scheme 13.6. in Sect. 13.1.3).

In 1985, *O'Malley et al*. published the total syntheses of *rac*-averufin (**103**) and *rac*-nidurufin (**104**) (*63*). These are both early precursors of the aflatoxins in their biosynthesis. Nidurufin (**104**) is the direct successor of averufin (**103**) and the direct precursor of versiconal hemiacetal acetate (**12**, see Scheme 2.1). Nidurufin (**104**) and averufin (**103**) are accessible by the same synthesis route; only the two last steps differ from each other (see Scheme 2.17). The first reaction was a double *Diels-Alder* reaction with dichloro-*p*-benzoquinone (**97**) and two equivalents of diene **98**. Then, three of the four alcohol functions were selectively MOM-protected (→ **99**). The remaining alcohol was converted into the allyl ether and then subjected to a reductive *Claisen* rearrangement, followed by MOM-protection of the redundant alcohol (→ **100**). By addition/elimination of PhSeCl, **101** was formed. Deprotonation of *t*-butyl 3-oxobutanoate, followed by reaction with **101** yielded the pivotal intermediate **102**. This could be converted into *rac*-averufin (**103**) by deprotection of the alcohols and decarboxylation at the side chain. The last step was a *p*-TsOH-catalyzed cyclization to give **103**. By treating **102** with *m*-CPBA, the double bond is epoxidized. *rac*-Nidurufin (**104**) was then formed by cyclization of this epoxide under acidic conditions.



Scheme 2.17 Total syntheses of averufin (**103**) and nidurufin (**104**). Reagents and conditions: a) THF, −78°C to rt, 2 h; then 120°C; then MeOH/HCl (aq.), reflux, 0.5 h, 50%; b) MOMCl, DIPEA, THF, 0.5 h, 88%; c) MOMCl, KO*t*-Bu, THF, 95%; d) allyl bromide, K_2CO_3, acetone, reflux, 12 h, 97%; e) $NaHCO_3$, $Na_2S_2O_4$, DMF/H_2O, 90°C, 89%; f) MOMCl, *t*-BuOK, THF, 91%; g) PhSeCl, CCl_4, rt; h) H_2O_2, pyridine, 0°C to rt, 2 h, 83% over two steps; i) NaH, *t*-butyl acetoacetate, DMSO, 1 h; then NaI, **101**, rt, 12 h, 70%; j) HOAc/H_2O, H_2SO_4 (cat.), 90°C, 3 h; k) *p*-TsOH (cat.), toluene, Δ, 50% over two steps; l) *m*-CPBA, $CHCl_3$, rt, 93%; m) HOAc/H_2O, H_2SO_4 (cat.), 90°C, 4 h; 69%

Other syntheses of *rac*-averufin (**103**) have been presented by *Townsend et al*. in 1981 and 1988, both *via* methoxymethyl-directed aryl metalation (*64*, *65*).

A later precursor of the aflatoxins, versicolorin A (**13**, see Scheme 2.1), has been synthesized by *Graybill et al*. in 1999. They also described the total syntheses of versicolorin B (**14**, see Scheme 2.1), versicolorin A hemiacetal (**105**), and 6-deoxyversicolorin A (**106**) (shown in Fig. 2.6) (*66*).

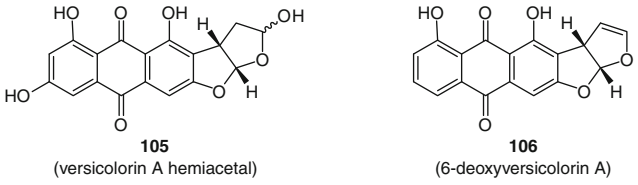
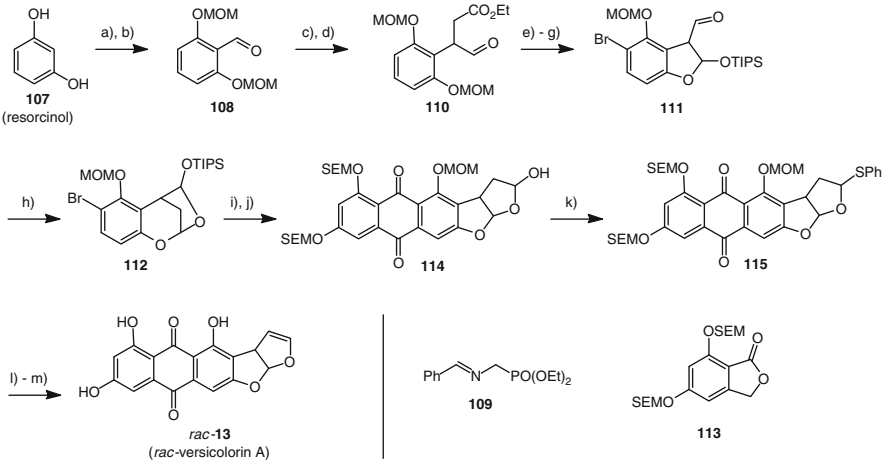


Fig. 2.6 Versicolorin A hemiacetal (**105**) and 6-deoxyversicolorin A (**106**), synthesized by *Graybill et al*.

The synthesis of *rac*-versicolorin A (**13**) is shown in Scheme 2.18. Resorcinol (**107**) was MOM-protected and formylated to yield **108**. *Horner-Wadsworth-Emmons* reaction with **109**, followed by deprotection and reaction with ethyl bromoacetate gave, after hydrolysis, phenyl acetaldehyde **110**. With TIPSOTf and triethylamine, cyclization occurred rapidly, followed by mono deprotection.

NBS brominated the aromatic ring at the *ortho*-position to the OMOM-group and DIBAL-H reduced the ethyl ester to give aldehyde **111**. Catalytic amounts of TIPSOTf promoted lactolization (→ **112**). After lithium-bromine exchange at **112**, reaction with lactone **113** gave a xanthone species, which reacted, after deprotection of the TIPS-group, to the five-ring species **114**. Transformation of the alcohol into thioether **115**, followed by global deprotection, oxidation, and pyrolysis gave *rac*-versicolorin A (**13**).



Scheme 2.18 Total synthesis of *rac*-versicolorin A (**13**) (*66*). Reagents and conditions: a) MOMCl, DIPEA, 81%; b) *n*-BuLi, DMF, 63%; c) *n*-BuLi, **109**, THF, −70°C, 1 h; then −78°C, **108**, 30 min; then 15°C; d) *n*-BuLi, −78°C; then −65°C, 2 h; then ethyl 2-bromoacetate, −78°C to rt, 66% over two steps; e) TIPSOTf, TEA, THF, 0°C, 82%; f) NBS, 77%; g) DIBAL-H, Et_2O, −95°C, 99%; h) TIPSOTf (cat.), CH_2Cl_2, −43°C, 5 min, 96%; i) LiTMP, **113**, −78°C; then −43°C, **112**, 2 h, 34%; j) TBAF, THF, −78°C to −20°C, 90%; k) 2-(phenylthio)isoindoline-1,3-dione, PBu_3, THF, −78°C to 0°C; then −78°C, **114**; then −2°C, 92%; l) HCl, HOAc, THF/H_2O, 65°C, 5 h, 97%; m) *m*-CPBA, $CHCl_3$, −15°C, 2 h; n) toluene, reflux, 45 min, 79% over two steps