

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).

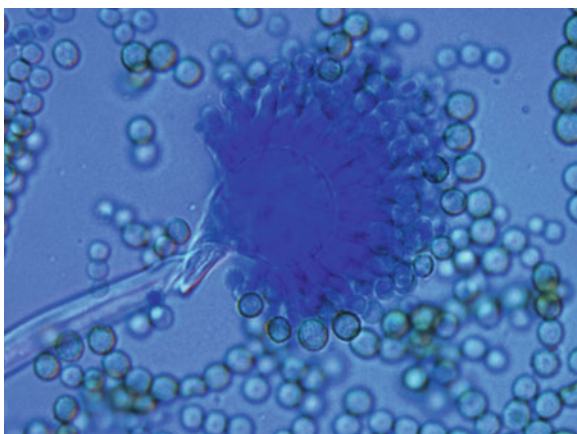


Fig. 2.1 *Aspergillus flavus* spores as seen under the light microscope under 600-fold magnification

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).

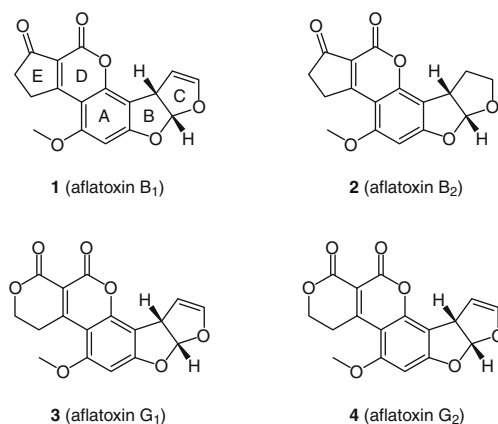


Fig. 2.2 The aflatoxins B₁, B₂, G₁, and G₂ (1–4)

The most widely examined aflatoxin is aflatoxin B₁ (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 B₂ (2), G₁ (3), and G₂ (4), which are shown in Fig. 2.2 (19). Their structures were revealed by the group of Büchi in 1963 (B₁ (1) and G₁ (3)) and 1965 (B₂ (2) and G₂ (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 B₁ (1) and B₂ (2), the so-called “milk-toxins”, M₁ (5) and M₂ (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 B₁ (1) and B₂ (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

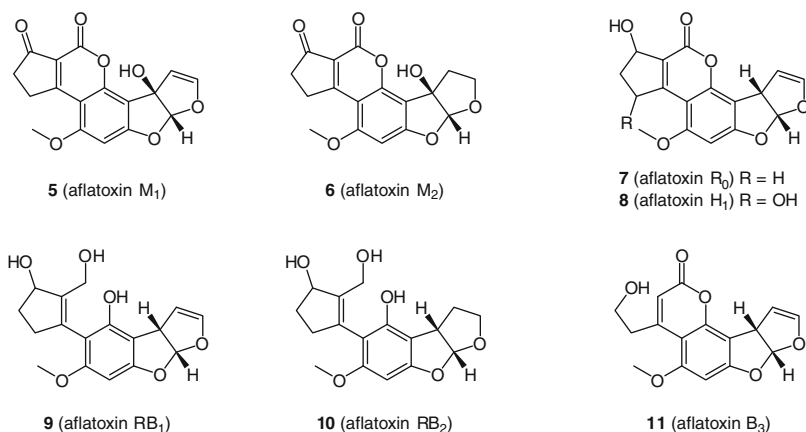
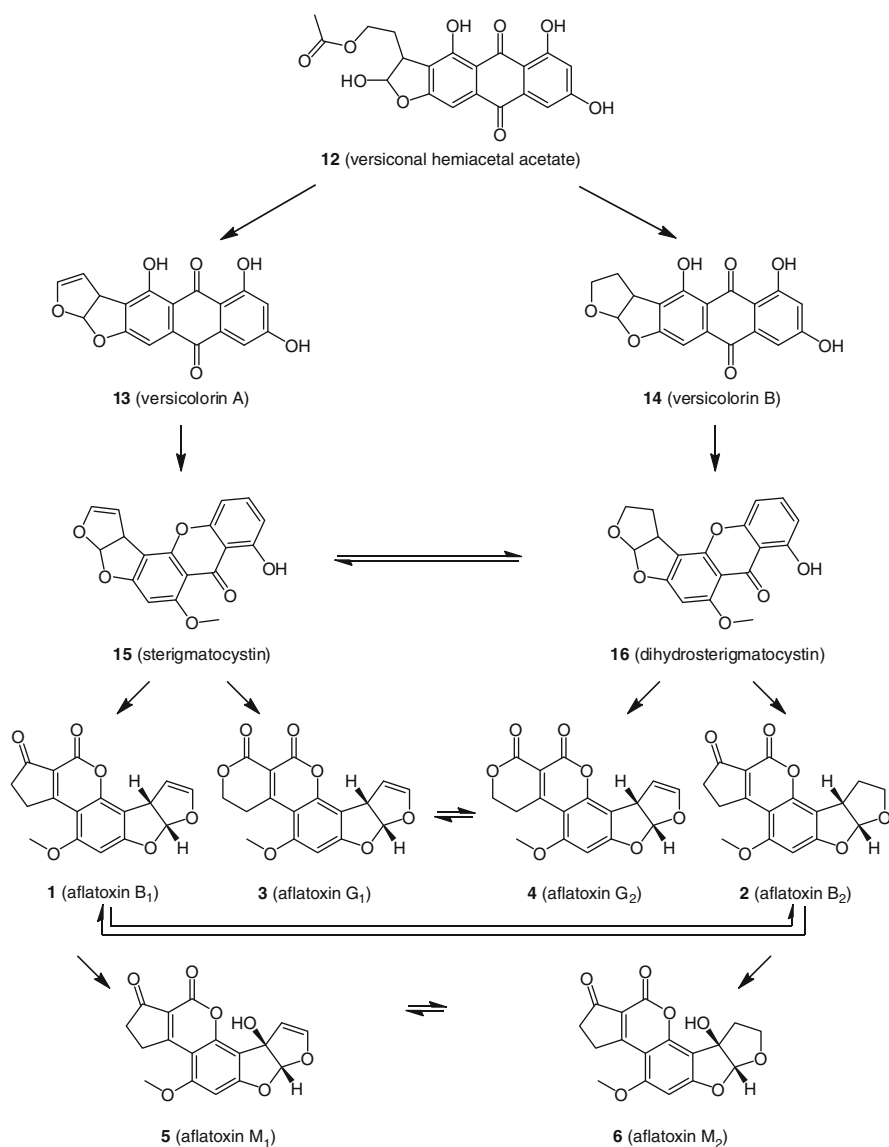


Fig. 2.3 Selected aflatoxins

a carbonyl group at ring E (R₀ (**7**), RB₁ (**9**), RB₂ (**10**), and H₁ (**8**)). They can be formed by microbial transformation or by chemical reduction with sodium borohydride (23, 24). In some aflatoxins, the D-ring (RB₁ (**9**), RB₂ (**10**)) or the E-ring (B₃ (**11**)) is opened. Aflatoxin B₃ (**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 G₁ (**3**) or B₁ (**1**) and the latter may then be transformed to aflatoxin M₁ (**5**). Aflatoxins B₂ (**2**) and G₂ (**4**) are formed from dihydrosterigmatocystin (**16**) and aflatoxin M₂ (**6**) is formed by conversion from B₂ (**2**). Pathways also exist to convert aflatoxin B₁ (**1**) to B₂ (**2**), M₁ (**5**) to M₂ (**6**), and G₁ (**3**) to G₂ (**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 B₁ (**1**) may be transformed to aflatoxin M₁ (**5**), representing a detoxification, since aflatoxin M₁ (**5**) is less active than aflatoxin B₁ (**1**) (see below) (27). However, a common metabolic process is diol formation at the double bond of the furan ring. The resultant aflatoxin B₁-2,3-diol is much more toxic than aflatoxin B₁ (**1**) itself. Accordingly, diol formation results from metabolic activation to a very toxic species (29).

Among the naturally occurring aflatoxins, aflatoxin B₁ (**1**) is the most acutely toxic representative, followed by aflatoxins G₁ (**3**), B₂ (**2**), and G₂ (**4**). This is shown by *LD*₅₀ values of one-day-old ducklings. While the *LD*₅₀ of aflatoxin B₁ (**1**) is 0.36 mg/kg, the corresponding value for aflatoxin B₂ (**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 G₁ (**3**) with that of B₁ (**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).

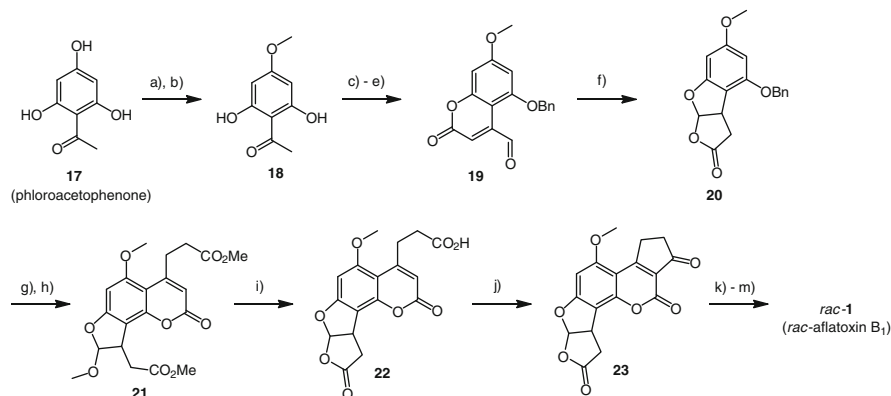
Besides their acute toxicity, aflatoxins are also highly carcinogenic. In fact, aflatoxin B₁ (**1**) is the most potent known liver carcinogen for mammals. It can not only induce tumors and metastases when directly injected, but also when it is given orally over a long period (13). Aflatoxins inhibit 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 B₁ (**1**), aflatoxin R₀ (**7**) is the most powerful mutagen, followed by aflatoxins M₁ (**5**), H₁ (**8**), B₂ (**2**), and G₂ (**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

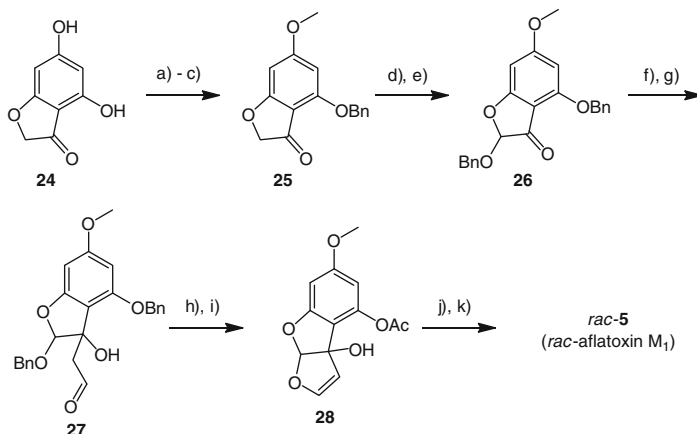
The group of *Büchi*, who also determined the structure and absolute configuration of several aflatoxins (20–22), achieved the first total synthesis of racemic aflatoxin B₁ (**1**) in 1966 (34, 35). They started from phloroacetophenone (**17**), which was converted in two steps into its monomethyl ether **18** (see Scheme 2.2). Selective monobenzoylation, followed by *Wittig* condensation and selenium dioxide oxidation gave the bicyclic aldehyde **19** in good yield.



Scheme 2.2 First total synthesis of aflatoxin B₁ (**1**), achieved by *Büchi et al.*. Reagents and conditions: a) Ac₂O, 110–165°C, 2 h, 40%; b) CH₂N₂, Et₂O/dioxane, rt; then HCl, MeOH, reflux, 8 h, 83%; c) BnBr, K₂CO₃, acetone, rt, 14 h, 82%; d) carbethoxymethylenetriphenylphosphorane, 170°C, 19 h, 72%; e) SeO₂, xylene, reflux, 5 h, 93%; f) Zn, HOAc, 100–120°C, 1.5 h, 80%; g) H₂, 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, H₂O, HCl (aq.), rt, 24 h, quant; j) (COCl)₂, CH₂Cl₂, 5°C to rt, 48 h; then AlCl₃, CH₂Cl₂, –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.), Ac₂O, 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 B₁ (**1**) in 13 steps and 0.9% overall yield from **17**.

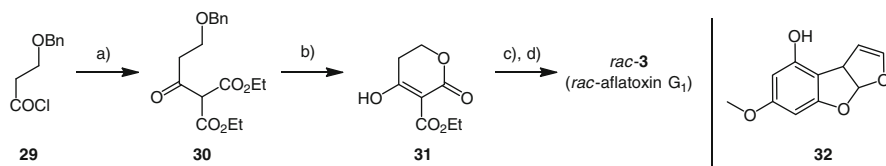
In 1969, Büchi *et al.* published the first total synthesis of racemic aflatoxin M₁ (**5**) (36). They started with the diol **24**, which was first dimethylated with dimethyl sulfate, then mono deprotected by aluminum chloride, and finally benzylated to afford species **25** (see Scheme 2.3).



Scheme 2.3 Total synthesis of racemic aflatoxin M₁ (**5**) by Büchi *et al.* Reagents and conditions: a) Me₂SO₄, K₂CO₃, dimethoxyethane, reflux, 3 h, 79%; b) AlCl₃, CH₂Cl₂, reflux, 1.25 h; then HCl, reflux, 64%; c) BnBr, K₂CO₃, dimethoxyethane/DMF, reflux, 74%; d) Me₃NPhBr₃, THF, 88%; e) CaCO₃, BnOH, Δ, 1.5 h, 65%; f) allylmagnesium bromide, THF/Et₂O, 0°C, 10 min; g) NaIO₄, OsO₄, NaHCO₃, dioxane/water, rt, 1 h, 63% over two steps; h) H₂, Pd/C, NaOAc, Ac₂O/benzene, rt, 1.5 h, 27%; i) toluene, 450°C, 73%; j) NaHCO₃, MeOH/H₂O, rt, 0.75 h, 94%; k) 2-carboxy-3-bromocyclopent-2-enone, NaHCO₃, ZnCO₃, CH₂Cl₂, 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 M₁ (**5**) in 11 linear steps from **24** and 0.7% overall yield.

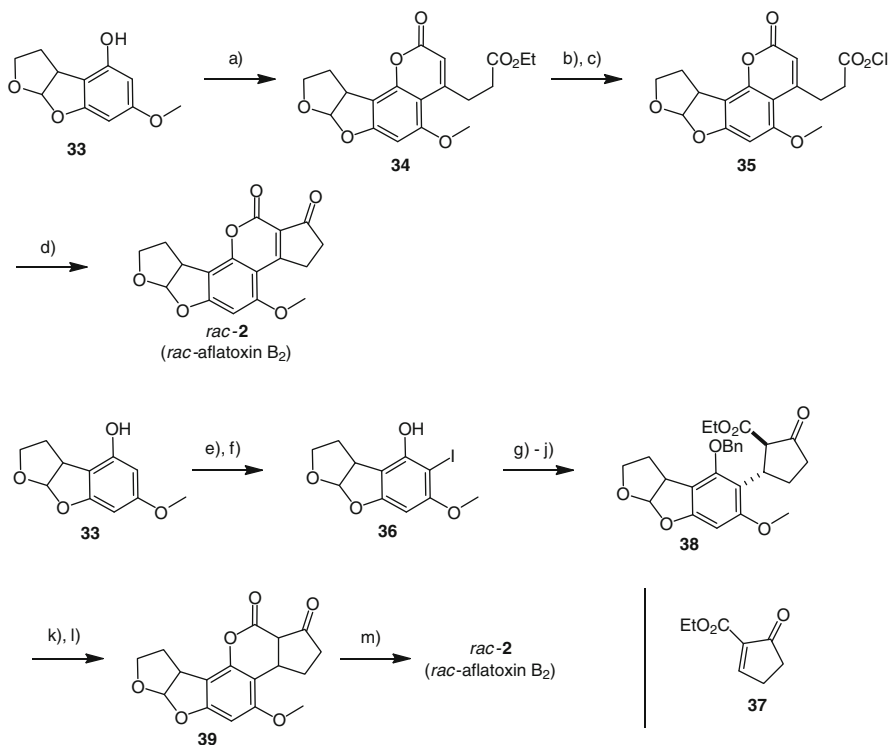
One year later, in 1970, Büchi and Weinreb presented a total synthesis of racemic aflatoxin G₁ (**3**) and an improved synthesis of aflatoxin B₁ (**1**) (37). The synthesis of **1** involved the same coupling with a cyclopentenone as described above for the total synthesis of aflatoxin M₁ (**5**) (see 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 G₁ (**3**). Reagents and conditions: a) diethylmalonate, Mg, ethanol/CCl₄, 0°C; then Et₂O, reflux, 3 h; then **29**, Et₂O, rt, 2 h, 97%; b) H₂, Pd/C, EtOAc, rt, 2 h, 64%; c) (COBr)₂, benzene, rt, 96%; d) **32**, ZnCO₃, LiI, CH₂Cl₂, rt, 3 h; then reflux, 7 h; then rt, 14%

The synthesis of aflatoxin G₁ (**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 G₁ (**3**). Different syntheses of the tricyclic **32** are presented in Sect. 2.3.2.

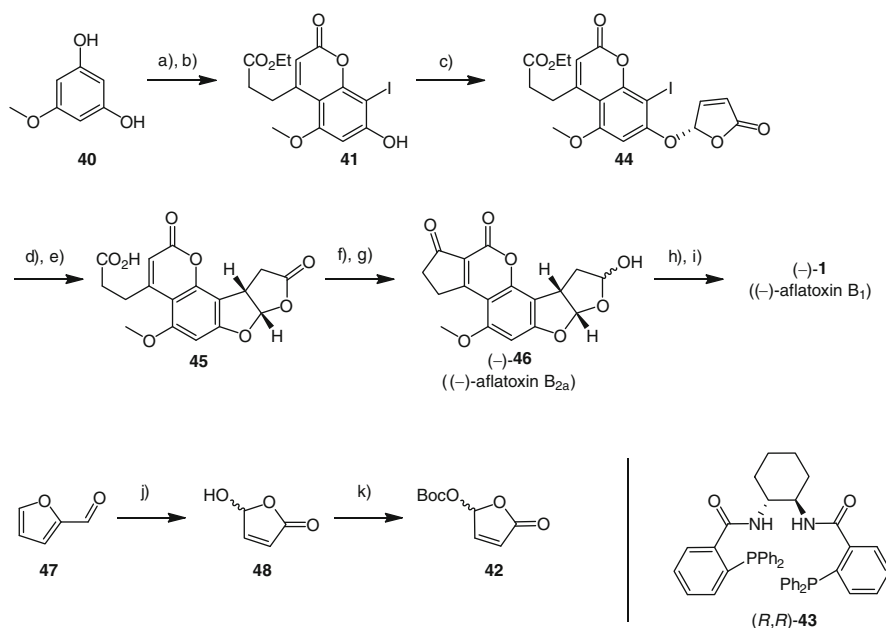
Aflatoxin B₂ (**2**) was first synthesized by *Roberts et al.* in 1968 (38). They started from the tricyclic compound **33**, for which the synthesis is described in Sect. 2.3.1. *Pechmann* condensation with diethyl β-oxoadipate generated the lactone **34**. Hydrolysis of the ethyl ester, followed by acid chloride formation with oxalyl chloride, gave **35**. This was used without further purification for a *Friedel-Crafts* acylation reaction to yield racemic aflatoxin B₂ (**2**). The synthesis is presented in Scheme 2.5, which also shows another total synthesis of aflatoxin B₂ (**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 B₂ (**2**) was achieved with DDQ.



Scheme 2.5 Syntheses of aflatoxin B₂ (**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, CH₂Cl₂; d) AlCl₃, CH₂Cl₂, −5°C, 3 h, 38% over two steps; e) Me₃BnNiCl₂, MeOH/CH₂Cl₂; f) NaH, 0°C; then *n*-BuLi, −100°C, 15 min, 70%; g) BnBr, K₂CO₃; 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) H₂, Pd/C, EtOAc, rt, 9 h, 200 psi; l) TFA, CH₂Cl₂, rt, 60% over two steps; m) DDQ, dioxane, rt, quant

2.2.2 Enantioselective Total Syntheses of Aflatoxins

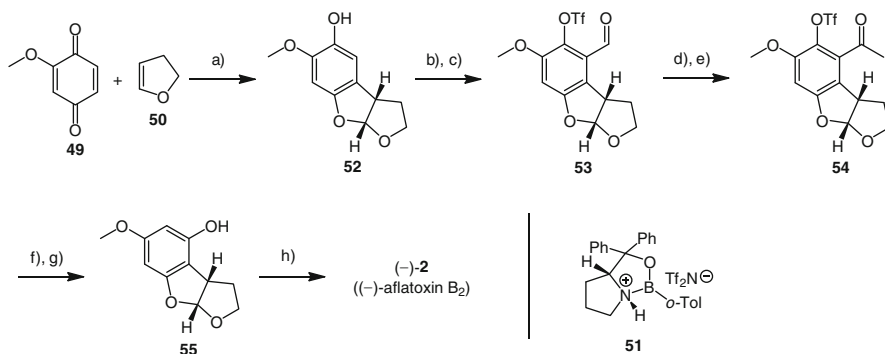
In 2003, *Trost* and *Toste* presented the first enantioselective total synthesis of aflatoxins B₁ (**1**) and B_{2a} (**46**) (40, 41). In Scheme 2.6, their synthesis is shown. The starting material for this sequence is catechol **40**. A *Pechmann* condensation with diethyl β-oxoadipate and iodination with iodine chloride gave the lactone **41**.



Scheme 2.6 Enantioselective total synthesis of (–)-aflatoxin B_{2a} (**46**) and (–)-aflatoxin B₁ (**1**). Reagents and conditions: a) diethyl β-oxoadipate, HCl, ethanol, rt, 3 d, 47%; b) ICl, CH₂Cl₂, rt, 30 min, 92%; c) **42**, Pd₂dba₃•CHCl₃, (*R,R*)-**43**, tetrabutylammonium chloride, CH₂Cl₂, rt, 12 h, 89%; d) (CH₃CN)₂PdCl₂, NEt₃, DMF, 60°C, 1 h, 93%; e) HCl, HOAc, H₂O, rt, 2 d, quant; f) Sc(OTf)₃, LiClO₄, CH₃NO₂, 60°C, 4 h, 32%; g) DIBAL-H, CH₂Cl₂, –78°C, 1 h, 57%; h) Ac₂O, HOAc, rt, 20 h; i) 240°C, 15 min, 24% over two steps; j) Rose Bengal, O₂, MeOH, 450 W Hg lamp, 8 h; k) Boc₂O, 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*-annulated rings are favored. Scandium(III)-mediated cyclization and reduction of the lactone with DIBAL-H yielded (–)-aflatoxin B_{2a} (**46**). It was acetoxyated and then pyrolyzed to give (–)-aflatoxin B₁ (**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 B₂ (**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 B₂ ((–)-**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 B₂ (**2**). Reagents and conditions: a) **51**, CH₂Cl₂/CH₃CN, -78°C to rt, 7 h, 65%, 99% *ee*; b) hexamethylenetetramine, HOAc, 110°C, 48 h, 40%; c) DMAP (cat.), pyridine, Tf₂O, CH₂Cl₂, -20°C to 0°C, 80%; d) MeMgBr, THF, -20°C, 2 h; e) *DMP*, CH₂Cl₂, 0°C to rt, 85% over two steps; f) TFAA, urea·H₂O, CH₂Cl₂, rt, 63%; g) Raney-Ni, H₂, MeOH, rt, 3 h, 60%; h) NaHCO₃, ZnCO₃, ethyl 2-bromo-5-oxocyclopent-1-enecarboxylate, CH₂Cl₂, rt, 20 h, 36%

2.3 Syntheses of Aflatoxin Building Blocks

2.3.1 Syntheses of Building Blocks for Aflatoxins B₂ and G₂

There are many different syntheses for the important building block **33** (Fig. 2.4). From this molecule, one can easily build aflatoxins B₂ (**2**) and G₂ (**4**) by the reactions presented in Sect. 2.2.

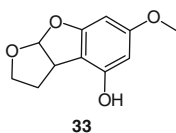
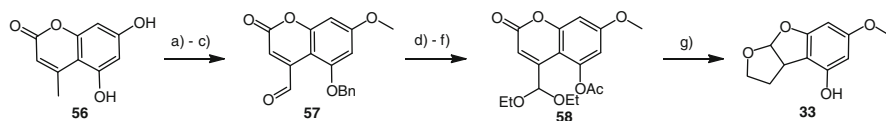


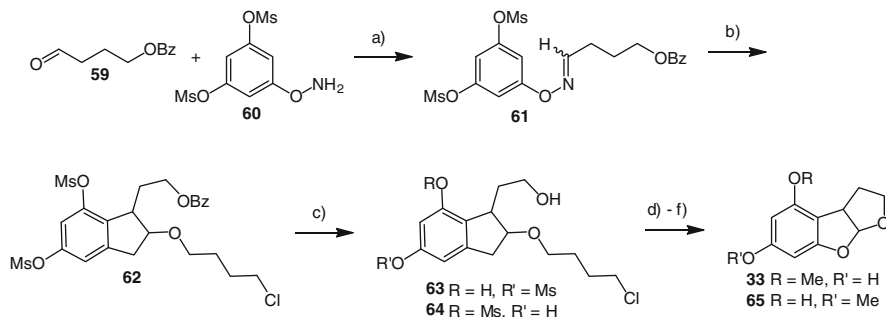
Fig. 2.4 Building block **33** for aflatoxins B₂ (**2**) and G₂ (**4**)

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 (\rightarrow **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 , $(\text{EtO})_3\text{CH}$, 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%

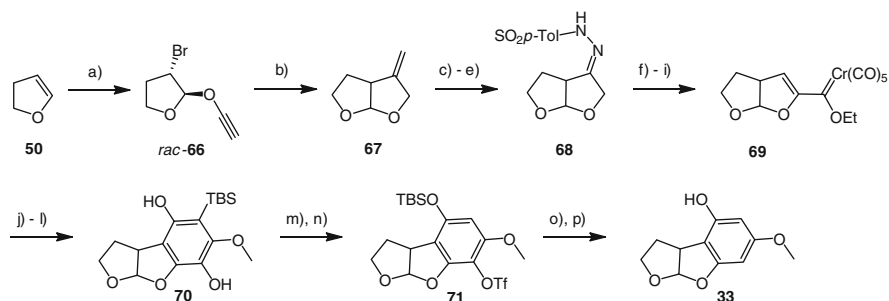
A straightforward access to **33** in six steps and 49% overall yield was published by *Castellino and Rapoport* in 1985 and is shown in Scheme 2.9 (44). The first step was an imine formation (\rightarrow **61**). By heating under acidic conditions, an oxaza-*Cope* rearrangement occurred, which, after 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) $\text{LiOH}\cdot\text{H}_2\text{O}$, 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 (\rightarrow **70**). Pyrolysis gave the protected alcohol and the remaining free alcohol was protected as a triflate (\rightarrow **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*-Tol SO_2NHNH_2 , THF, 79%; f) Na, triglycol, 120°C , 73%; g) *t*-BuLi, THF, -78°C ; h) $\text{Cr}(\text{CO})_6$; i) Et_3OBF_4 , 52% over three steps; j) *t*-butyl(methoxyethyl)dimethylsilane, THF, 80°C , 31%; k) CAN, $\text{H}_2\text{O}/\text{CH}_3\text{CN}$, 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). *Mitra et al.* synthesized **74** in the same way as *Snider et al.* (51).

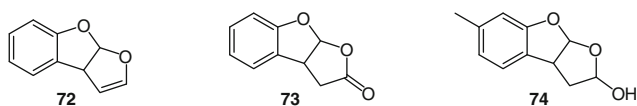
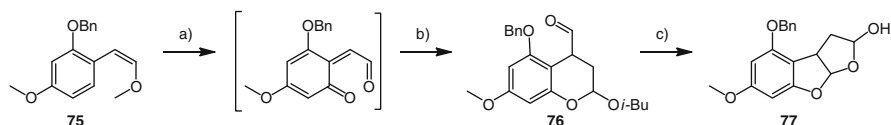


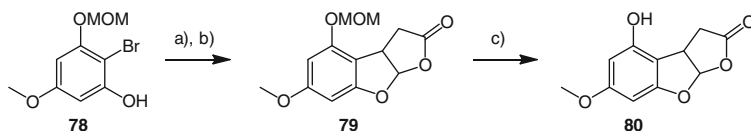
Fig. 2.5 Model systems for aflatoxin precursors

Matsumoto and *Kuroda* presented a short and elegant synthesis for an aflatoxin B₁ 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 (\rightarrow **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) ¹O₂; b) *i*-butyl vinyl ether, 39%; c) H₂SO₄ (cat.)

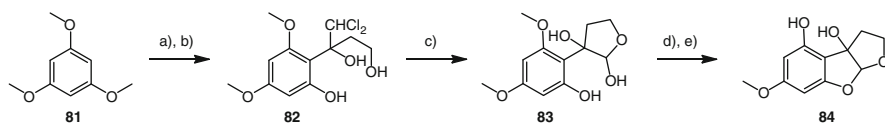
In 1988, *Sloan et al.* presented a building-block synthesis *via* radical-induced ring closure (53). The aromatic alcohol **78** was first substituted on 5-bromofuran-2(5*H*)-one, then an intramolecular, radical 1,4-addition formed **79**. Removal of the MOM-protecting group then gave **80**. The synthesis is shown in Scheme 2.12. From this intermediate, *Büchi et al.* described the synthesis of aflatoxin B₁ (**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, K₂CO₃, acetone, reflux; b) Bu₃SnH, AIBN, benzene, reflux; c) 9-BBN-Br, CH₂Cl₂, -78°C to 0°C, 1.5 h

2.3.3 Synthesis of a Building Block for Aflatoxin M₂

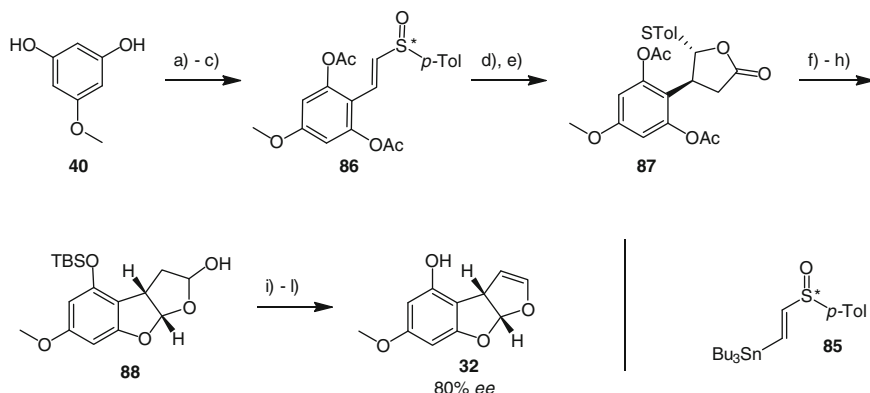
For aflatoxin M₂ (**6**), the required building block has been synthesized by *Kraus and Wang*, as shown in Scheme 2.13 (56). The starting material, 1,3,5-trimethoxybenzene (**81**), was first acylated and mono-demethylated *in situ*, then a 1,2-addition to the ketone provided **82**. Under basic conditions, 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 M₂ (**6**) can be achieved according to the protocol of *Büchi* for the synthesis of aflatoxin M₁ (**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) $BF_3 \cdot OEt_2$, 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 (\rightarrow **86**). Dichloroketene lactonization under reductive conditions followed by zinc-promoted dechlorination gave the major diastereomer **87**.

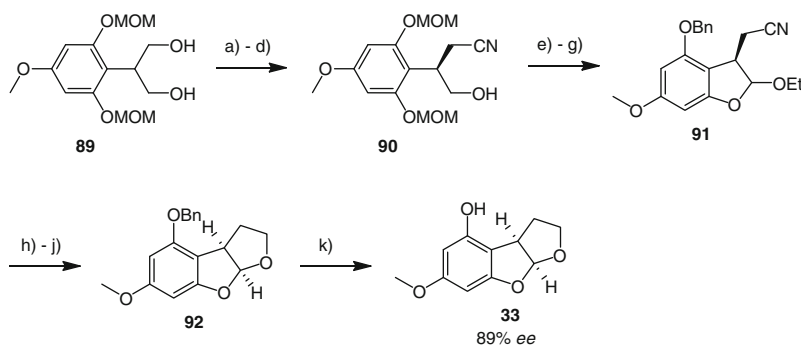


Scheme 2.14 Enantioselective synthesis of a building block (**28**) for aflatoxin B_1 (**1**). Reagents and conditions: a) $AcCl$, pyridine, 98%; b) $HgO \cdot HBF_4 \cdot SiO_2$, I_2 , 49%; c) **85**, $Pd(0)$, PPh_3 , toluene, reflux, 65%; d) $Zn(Cu)$, Cl_3CCOCl , THF, $-50^\circ C$; e) Zn , $HOAc$, 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 , $-78^\circ C$; k) pyridine, toluene, $110^\circ C$; l) CsF , CH_3CN , $0^\circ C$, 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 B₂ (**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 (\rightarrow **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 (\rightarrow **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 B₂ (**2**) according to *Büchi's* or *Robert's* conditions (37, 38).

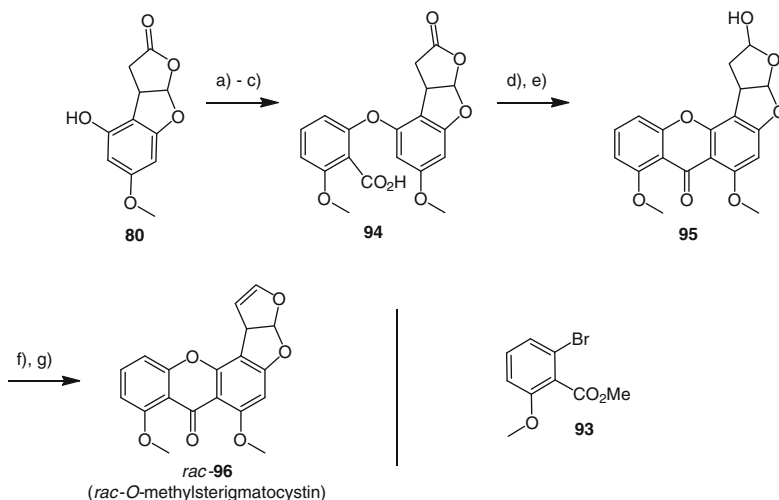


Scheme 2.15 Enantioselective synthesis of the aflatoxin B₂ building block **33**. Reagents and conditions: a) Lipase AL, vinyl acetate, Et₂O, rt, 72%, 89% *ee*; b) MsCl, DIPEA, DMAP, CH₂Cl₂, 89%; c) KCN, 18-Crown-6, DMSO, 72%; d) LiOH, THF/H₂O, 83%; e) TPAP, NMO, 4 Å MS, CH₂Cl₂; f) HCl, HC(OEt)₃, EtOH; g) BnCl, K₂CO₃, DMF, 50% over three steps; h) KOH, EtOH/H₂O; i) BH₃•SMe₂, THF; j) *p*-TsOH, CH₂Cl₂, 43% over three steps; k) 1,4-cyclohexadiene, 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 B₁ (**1**) and G₁ (**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.

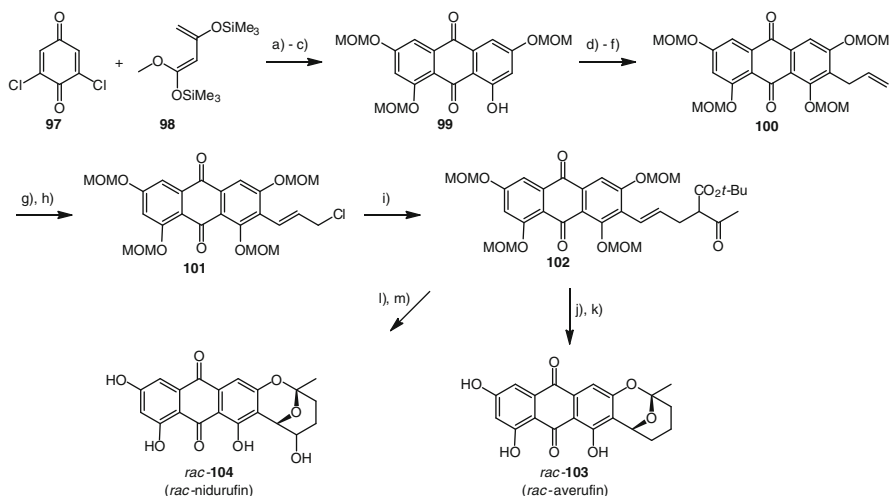


Scheme 2.16 Synthesis of *rac*-*O*-methylsterigmatocystin (**96**). Reagents and conditions:

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) $(\text{COCl})_2$, 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 (\rightarrow **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 (\rightarrow **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 $_2$ CO $_3$, acetone, reflux, 12 h, 97%; e) NaHCO $_3$, Na $_2$ S $_2$ O $_4$, DMF/H $_2$ O, 90°C , 89%; f) MOMCl, t -BuOK, THF, 91%; g) PhSeCl, CCl $_4$, rt; h) H $_2$ O $_2$, 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 $_2$ O, H $_2$ SO $_4$ (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 $_2$ O, H $_2$ SO $_4$ (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).

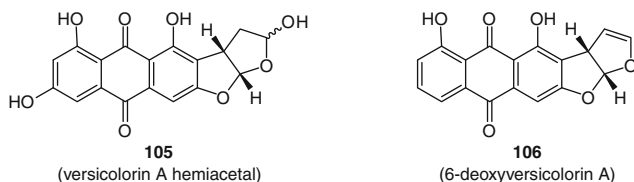
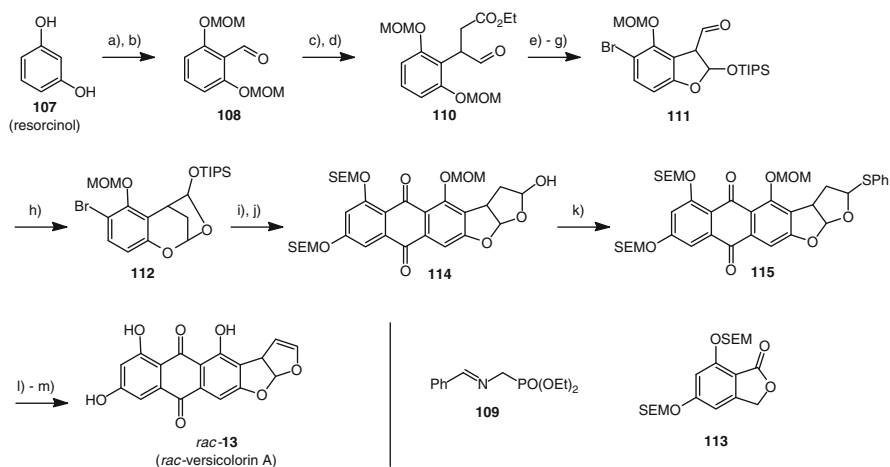


Fig. 2.6 Versicolorin A hemiacetal (**105**) and 6-deoxyversicolorin A (**106**), synthesized by Graybill *et al.*

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 (\rightarrow **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

The Chemistry of Mycotoxins

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