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Total Synthesis of the Aristolochic Acids, Their Major Metabolites, and Related Compounds

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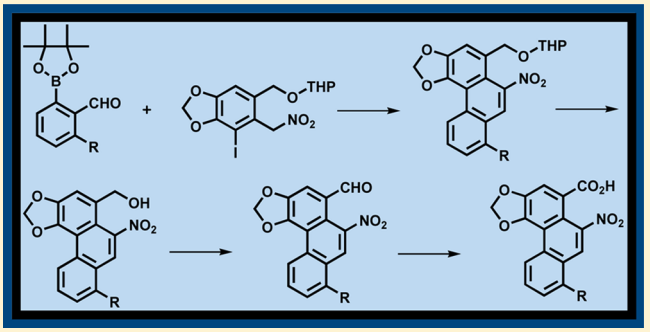
Supporting Information

ABSTRACT: Plants from the *Aristolochia* genus have been recommended for the treatment of a variety of human ailments since the time of Hippocrates. However, many species produce the highly toxic aristolochic acids (AAs), which are both nephrotoxic and carcinogenic. For the purposes of extensive biological studies, a versatile approach to the synthesis of the AAs and their major metabolites was devised based primarily on a Suzuki–Miyaura coupling reaction. The key to success lies in the preparation of a common ring-A precursor, namely, the tetrahydropyranyl ether of 2-nitromethyl-3-iodo-4,5-methylendioxybenzyl alcohol (**27**), which was generated in excellent yield by oxidation of the aldoxime precursor **26**. Suzuki–Miyaura coupling of **27** with a variety of benzaldehyde 2-boronates was accompanied by an aldol condensation/elimination reaction to give the desired phenanthrene intermediate directly. Deprotection of the benzyl alcohol followed by two sequential oxidation steps gave the desired phenanthrene nitrocarboxylic acids. This approach was used to synthesize AAs I–IV and several other related compounds, including AA I and AA II bearing an aminopropyloxy group at position-6, which were required for further conversion to fluorescent biological probes. Further successful application of the Suzuki–Miyaura coupling reaction to the synthesis of the *N*-hydroxyaristolactams of AA I and AA II then allowed the synthesis of the putative, but until now elusive, *N*-acetoxy- and *N*-sulfonyloxy-aristolactam metabolites.



INTRODUCTION

Various species of *Aristolochia* have been used as herbal remedies since the time of Hippocrates to treat a variety of human conditions and diseases.[1] A popular use has been in childbirth,[2] and until around 1980, these herbs were recommended by physicians, especially in the United Kingdom, to women at term. In searching for antitumor agents, Kupchan and Doskovich[3] examined aristolochic acid I (AA I, **1**), the principal chemical constituent of *Aristolochia indica*, but found that it was ineffective as an anticancer agent in mice. Jackson and his associates[4] found AA I to be nephrotoxic in human clinical trials, and Pezzuto et al.[5] subsequently confirmed these results but also observed the substance to be mutagenic. In 1993, a report from Vanherweghem and his associates[6,7] described an unfortunate event at a Belgian clinic where more than 1500 women were given pills as part of a slimming regimen that contained a Chinese herbal product derived from *Aristolochia fangchi*. More than one hundred developed a serious nephropathy characterized as a rapidly progressive, renal, interstitial fibrosis. Shortly thereafter, Cosyns and his colleagues[8,9] reported that patients suffering from aristolochia-induced nephropathy were also at risk for urothelial cancer. This drew attention to a similar nephropathic condition that was known to be endemic along the banks and tributaries of the Danube River but particularly in Croatia. In many of the villages there, a substantial number of the inhabitants suffer from a debilitating ailment[10] characterized by a renal fibrosis that is followed by a urothelial malignancy in approximately 50% of the cases.[8,11] The diseased state was termed Balkan endemic nephropathy (BEN), but its close identity in both morphological and clinical patterns to the outbreak in Belgium, known at the time as Chinese herbs nephropathy (CHN), led Cosyns[12] to suggest that they likely had a similar origin. Both diseased states are now termed aristolochic acid nephropathy (AAN).

Worldwide population exposure to aristolochic acids (AAs) is extensive. In China, where the use of herbal remedies is pervasive, the number of AAN cases is suspected to be very large despite a governmental ban on the use of *Aristolochia* species. Because of the widespread use of herbal products in Taiwan, a very large number of cases of AAN have been reported there recently,[13] but patients having AAN-like symptoms have been seen in many countries, best documented in Spain,[14] the United Kingdom,[15] and Japan.[16] The epidemiology, diagnosis, and management of AAN have recently been reviewed by Gökman and Lord in association with other eminent researchers in the field.[17]

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Aristolochia fangchi contains a mixture of AAs, the suspected AAN-etiologic agents, whose structures were first identified by Pailer and his associates.[18,19] Using a mixture of AA I (**1**) and AA II (**2**), it proved possible to reproduce the main features of the disease in rodents,[20,21] thus removing any doubts that the aristolochic acids are responsible for AAN in humans. Evidence that tumor development also could be ascribed to these acids rests on the isolation of DNA adducts from renal tissue.[22,23] These adducts have been identified[24] as **3** and **4** (Figure 1),

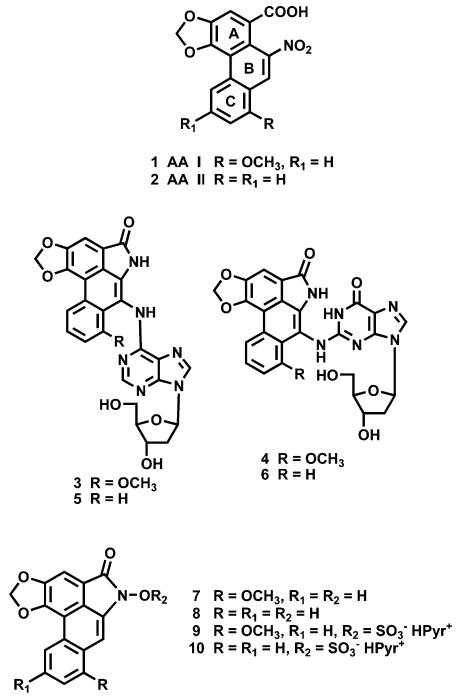

Figure 1. Chemical structures for the aristolochic acids, their DNA adducts, and selected metabolites.

derived from metabolites of AA I,[25] and the corresponding adducts, **5** and **6**, derived from AA II. In both cases, the adenosine adducts are dominant. All of these adducts appear to arise by the same metabolic pathways that are followed by most carcinogenic amines and nitro compounds,[26,27] in which the intermediate *N*-hydroxyamine metabolites (in the cases under discussion, the *N*-hydroxylactams, **7** and **8**) are the purported pro-carcinogens. Surprisingly, in the case of the AAs, the expected C-8 purine metabolic adducts, which are normally found with many of the known nitro- and amino-polyaromatic hydrocarbons, are not observed, and only the products of adduction at the exocyclic amino groups of these nucleobases are found.

In this current article, we record the total syntheses of the naturally occurring AAs I–IV together with a series of analogues intended to support advanced etiologic studies of AAN. We discuss some of the difficulties associated with their chemistry and define why we adopted a "total synthesis" route rather than employing the natural products. In addition, we have synthesized the proposed penultimate AA metabolites (Figure 1), namely, the *N*-sulfonyloxy AA-lactams as their pyridinium salts, respectively, **9** and **10**, and the corresponding putative metabolites, the *N*-acetoxy AA-lactams **39** and **40** (Scheme 6), together with two AAs having a 3-aminopropyloxy linker at the 7-position, namely **11** and **12** (Figure 2). The latter are precursors for the preparation (to be reported later) of derivatives containing a biological marker, either biotin or a fluorescent probe.

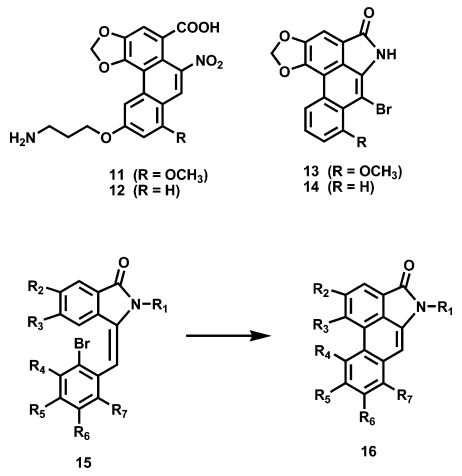

Figure 2. Chemical structures for synthetic intermediates.

RESULTS AND DISCUSSION

Synthetic Chemistry of AA I and AA II, Their Congeners, Related Metabolic Products, and Protein Probe Substances. AA I and AA II occur together naturally in many *Aristolochia* species and are available commercially as a mixture. However, they cannot be separated by crystallization, and their separation by column chromatography is a tedious procedure. By contrast, the corresponding methyl esters can be separated chromatographically, but attempts to hydrolyze the esters returned very poor yields of the parent acids. This unusual result is a reflection of the steric strain (the aromatic equivalent of 1,3-allylic strain[28]) that exists between the nitro and carboxyl functions and parallels identically the group compression that exists in 8-nitro-1-naphthoic acid (NPA). This latter acid cannot be esterified by the standard Fischer–Speier method, and its methyl ester undergoes substantial substitution of the nitro group when basic hydrolysis is attempted. Similarly, attempts to form the acid chloride of NPA with $SOCl_2$ leads mainly to 8-chloro-1-naphthoic acid after hydrolysis and lesser amounts of 5,7-dichloronaphthostyril.[29] Besides these problems, the positions of the electron-donating groups already present in the AAs restrict their versatility in further desirable aromatic substitution reactions. On the basis of such aberrant chemistry, the possibility of using the naturally occurring AAs in further synthetic manipulations seemed limited. We elected to attempt to develop a general *de novo* total synthesis of the AAs and their principal metabolites (a) to allow a broader structure–activity study than would be possible with the natural substances, (b) to permit the metabolic fate of these substances to be examined under controlled conditions, and (c) to probe for proteins involved in the nephrotoxicity of AA I.

The synthesis of AA I has previously been reported by Kupchan and Wormser,[30] while the synthesis of AA IV was achieved by Pailer and his associates.[31] Both methods are similar and rely on a photochemical coupling step that is impracticable for larger-scale development. In addition, too many steps are involved, yields at some stages are low (in particular, the final basic hydrolysis of AA I methyl ester gives AA I in only 6% yield[30]), and the route lacks versatility and

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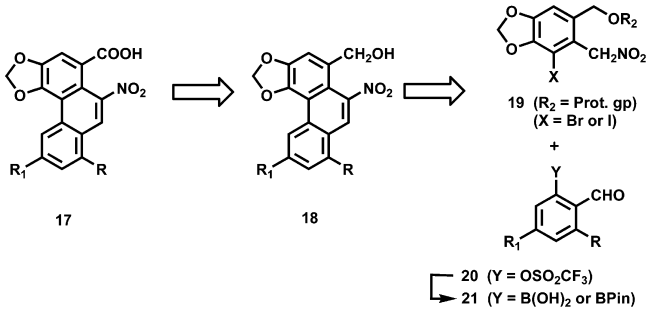
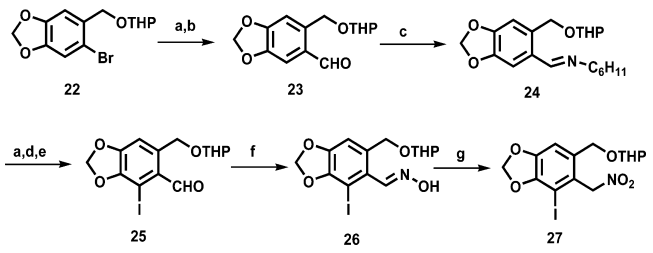
would not be easily adaptable to the synthesis of the 7-bromoaristolactams **13** and **14** that we saw as the critical intermediates in our synthetic approach to the DNA adducts **3**–**6**. The work of Castedo and his associates,[32] which uses an internal benzyne reaction to construct the aristolactam framework, did not seem applicable to the synthesis of the AAs themselves. Similarly, the methodology, principally evolved by Couture et al.[33,34] for the synthesis of a series of the related, naturally occurring aristolactams, was not easily adaptable to the synthesis of the AA metabolites **7**–**10**. Although their methods are elegant, they are relatively inefficient because (a) the typical isoindolenone intermediate **15** is frequently obtained as a mixture of geometric isomers and (b) the cyclization to give the required penultimate lactam **16** using the Bu_3SnH/AIBN catalyst is problematic on a larger scale. We envisaged that a more efficient synthetic route (see Scheme 1 for the retrosynthetic approach) might be possible, first by linking the two principal precursors, namely, **19** and **21**, of the aromatic rings A and C by means of a Suzuki–Miyaura reaction, which together with a likely sequential condensation would lead directly to the OH-protected form of phenanthrene **18**, avoiding the problem of geometrical isomerism. In any event, this procedure proved to be both efficient and versatile for the synthesis of the aristolochic acids and their lactams and by similar chemistry for the synthesis of their *N*-hydroxyaristolactam metabolites **7**–**10**. The efficient synthesis of a series of AA-related lactams employing a similar Suzuki–Miyaura strategy has also been described more recently by Kim et al.[35,36]

Scheme 1



Comprehensive Methods of Synthesis of the Aristolochic Acids. *Ring-A: Component 19.* The key to our general synthetic approach lies in the efficient synthesis of an α-nitrotolyl compound (ring-A precursor) represented generically by **19**. This was realized in the iodo compound **27** whose synthesis was accomplished in the five steps outlined in Scheme 2. Lithiation of **22** at −75 °C followed by quenching with dimethylformamide gave **23** in 75% yield.[37] The latter was condensed with cyclohexylamine to give **24** (97% yield), which, when treated with butyl lithium[38] followed by iodine both at −70 °C, led to the ortho-iodinated compound **25** that was still contaminated by small amounts of unreacted starting material **24**. Whereas treatment of the mixture with hydroxylamine acetate afforded an impure solid, the pure oxime **26** could be isolated cleanly by trituration with di-isopropyl ether (54% overall yield from compound **24**). Oxidation of **26** to **27** proved challenging. Although we tried many of the usual procedures[39−44] to achieve this transformation, we were successful only with the rarely used benzo-peroxo-molybdenum complex,[45] which under the neutral conditions described by Ballisteri et al.[46] gave the desired compound **27** in 67% yield. The overall procedure allowed for the preparation of **27** in ∼50 g batches.

Scheme 2[a]

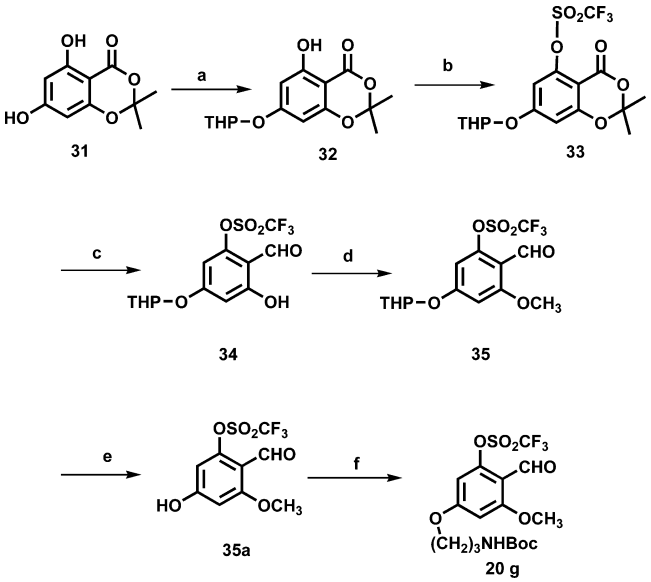


[a]Reagents: (a) BuLi; (b) DMF; (c) cyclohexylamine; (d) I_2; (e) H_3O^+; (f) $HONH_2$/HOAc; (g) peroxomolybdate.

It is worth noting that the more logical use of a carboxylic acid or ester in place of the protected benzyl alcohol in **19** did not prove feasible because of the formation of cyclic benzoxazinone products at the later oxime stage. Similarly, when we began the synthesis with a methyl group in place of CH_2OH in the sequence, it was not possible to functionalize the former either by halogenation or oxidation at the phenanthrene stage. Thus, we employed the protected benzyl alcohol as a surrogate for the carboxylic acid with the intent of generating the acid later in the sequence.

Ring C: Component 21. The required triflates (**20b**–**f**) were prepared from the corresponding phenols by standard methods or, in the case of the trifluoromethoxy analogue **20f**, by the method of Choshi et al.[47] The aminopropyl derivative **20g** was synthesized as illustrated in Scheme 3 by an adaptation of the procedures of Bajwa and Jennings[48] for the unambiguous preparation of substituted salicylaldehydes and the corresponding benzyl alcohols. Compound **20h** was prepared similarly starting from 2,4-dihydroxybenzaldehyde. The syntheses of the substituted phenyl-2-formyl boronates (**21b**–**f**), apart from the simplest analogue (**21a**: R = R_1= H) which is commercially

Scheme 3[a]



[a]Reagents: (a) DHP/H^+; (b) Tf_2O/Pyr; (c) DIBAL; (d) CH_3I/K_2CO_3; (e) CF_3COOH/MeOH; (f) $I(CH_2)_3NHBoc/K_2CO_3$.

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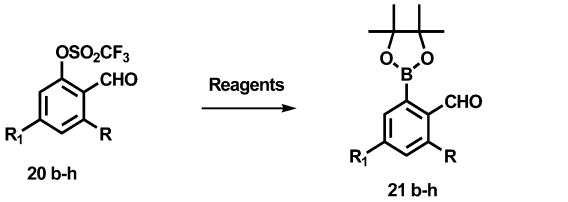
available, were all carried out by the known Pd-catalyzed/pinacolato diborane substitution reaction of the corresponding triflates (**20b–f**), again according to a procedure by Choshi et al.[47] (Scheme 4). Some of these boronates proved to be unstable to chromatography and were used directly in the succeeding Suzuki–Miyaura coupling reaction without further purification.

Scheme 4[a]



20 b-h 21 b-h

Substituents for 20/21; b (R = OCH_3, R_1 =H) c (R = R_1 = OCH_3)
d (R = H, R_1 = OCH_3) e (R = OCH_2OCH_3, R_1 = H)
f (R = OCF_3, R_1 = H) g (R= OCH_3, R_1 = $O(CH_2)_3NHBoc$)
h (R = H, R_1 = $O(CH_2)_3NHBoc$).

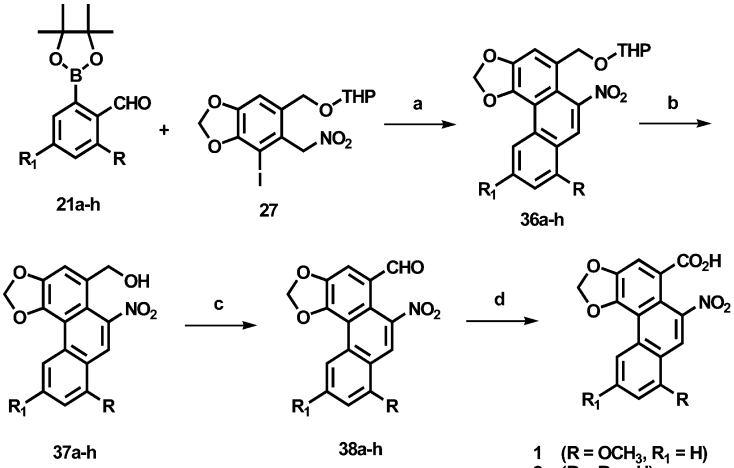
[a]Reagents: $B(Pin)_2/Pd(dppf)_2Cl_2/KOAc$.

Coupling of Ring Components A and C: Aristolochic Acid Synthesis. The coupling/condensation of the aldehydic borate esters **21a–h** with the phenylnitromethane **27** (Scheme 5) proceeded smoothly in all cases in aqueous dioxane at 95 °C using tetrakis(triphenylphosphine) palladium as the catalyst in the presence of cesium carbonate. Yields of products **36a–h** varied from 56 to 77% after chromatographic purification over silica gel. Removal of the tetrahydropyranyl group to give the alcohols **37a–h** was accomplished almost quantitatively by heating to boiling in methanol containing 0.1% sulfuric acid, then cooling immediately to room temperature.[49] However, all attempts to oxidize the very poorly soluble alcohols directly to the carboxylic acids **1**, **2**, and **1c–h** failed, no matter which common oxidant (e.g., CrO_3, chromyl chloride, NaOCl, $KMnO_4$, etc.) was used. Mostly, the aldehydes **38a–h** or decomposition products were obtained. The conversion was therefore accomplished in two steps, initially by oxidation to the aldehydes **38a–h** using either activated MnO_2 in acetone or CrO_3 in acetic acid, followed by a second oxidation with sodium chlorite in aqueous DMSO buffered by sodium dihydrogen phosphate.[50] This afforded all of the desired aristolochic acids (**1**, **2**, and **1c–h**) in acceptable overall yields. The physical data for the synthetic aristolochic acids that are also known natural products were identical to those reported in the literature.[51] In the cases of **1g** and **1h**, direct treatment with HCl generated **1i** and **1j** as their hydrochloride salts, suitable for further coupling with a dyestuff or a fluorescent probe.

Synthesis of Putative Metabolites of the Aristolochic Acids. As noted previously, carcinogenic aromatic amines and nitro compounds generally share a common metabolic pathway that ultimately leads to DNA adduction followed by mutations that can lead to tumor induction. In the case of the nitro-polyaromatic hydrocarbons, this involves phase I enzymatic reduction to a hydroxylamine, which is subsequently activated by phase II metabolism either by O-acetylation or O-sulfonylation. Solvolysis of these derivatives to generate the "ultimate carcinogens", namely, the intermediate nitrenium/carbenium ions, in the presence of DNA then leads to covalent adduction.[26,27] In the case of the aristolochic acids, this translates into the pathway shown in Scheme 6.

The synthesis of the metabolic derivatives of the AAs follows a pathway similar to that described above for the AAs, again using a Suzuki–Miyaura reaction to establish the phenanthrene framework. This required the synthesis of **46b** as the basis of ring-A and was accomplished as shown in the first three steps of Scheme 7. The coupling reactions of **46b** with either **21a** or **21b** to obtain **7** or **8** in good yield were accomplished directly by means of the Pd-based catalyst, [1,1-bis-(diphenylphosphino)ferrocenyl]dichloro-palladium(II), in the presence of potassium carbonate in dioxane/water at 100 °C.

Scheme 5[a]



21a-h 27 36a-h

37a-h 38a-h

1 (R = OCH_3, R_1 = H)
2 (R = R_1 = H)
1c (R = R_1= OCH_3)
1d (R = H, R_1=OCH_3)
1e (R = OCH_2OCH_3, R_1= H)
1f (R = OCF_3, R_1 = H)
1g (R = OCH_3, R_1 = $O(CH_2)_3NHBoc$)
1h (R = H, R_1 = $O(CH_2)_3NHBoc$
1i (R = OCH_3; R_1 = $O(CH_2)_3NH_2$)
1j (R = H; R_1 = $O(CH_2)_3NH_2$)

[a]Reagents: (a) $Pd(PPh_3)_4/Cs_2CO_3$, 95 °C; (b) H_3O^+; (c) MnO_2 or CrO_3/Pyr; (d) $NaClO_2/NaH_2PO_4$/DMSO/H_2O.

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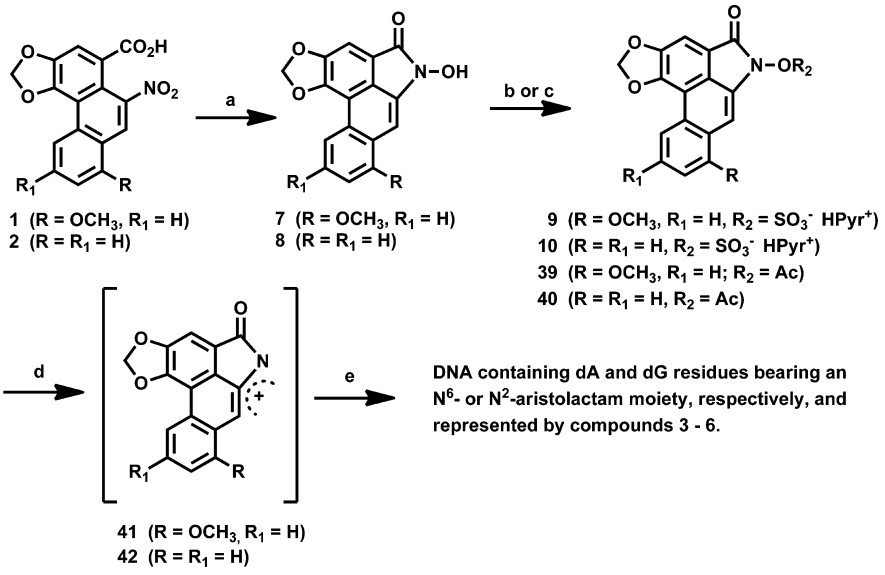
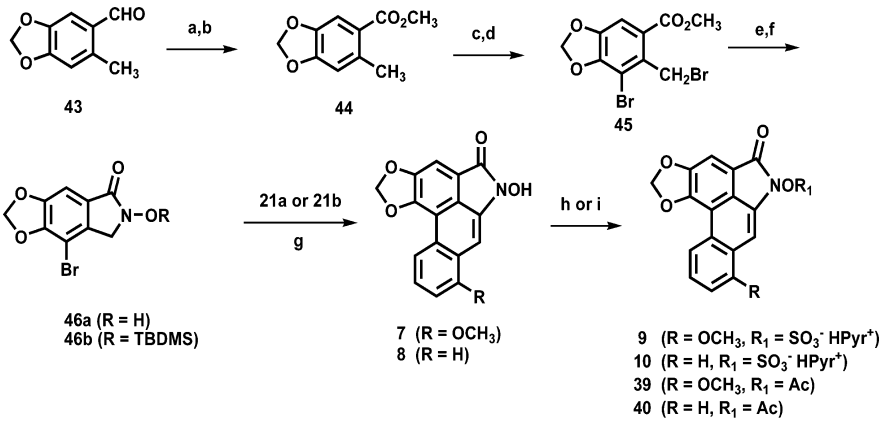
Scheme 6[a]



[a]Reagents: (a) NQOI; (b) NAT; (c) SULT1B1; (d) electrophile, e.g., H_3O^+; (e) DNA.

Scheme 7[a]



[a]Reagents: (a) $NaClO_2/NaH_2PO_4$; (b) $(CH_3O)_2SO_2/NaOH$; (c) $Br_2/HOAc$; (d) NBS; (e) $NH_2OH{\cdot}HCl/NaOH$; (f) TBDMSCl/Pyr; (g) $d(dppf)Cl_2/K_2CO_3$/dioxane/H_2O, 100 °C, 16 h; (h) SO_3/Pyr; (i) Ac_2O/Pyr.

Surprisingly, not only was the Suzuki–Miyaura coupling accomplished between the A and C ring components, but it was accompanied by the condensation of the aldehyde with the methylene of the lactam to form ring-B. Additionally, the TBDMS protecting group was lost from the hydroxylactam in a highly desirable cascade sequence that gave only one major product in each case. The completion of the syntheses of the four purported AA metabolites **9**, **10**, **39**, and **40** was straightforward by treatment of **7** or **8** with either sulfur trioxide/pyridine or acetic anhydride/pyridine, respectively. The hydroxylactams and the corresponding *O*-acetyl derivatives proved to be very stable in the solid state, in aqueous solution buffered to pH 6 and in 1 N hydrochloric acid for 18 h with no loss of integrity. However, the *O*-sulfonylpyridinium salts decompose in aqueous media (pH 6) but can be stored in the solid state almost indefinitely at −20 °C. Biological studies with these substances will be reported separately.[52]

CONCLUSIONS

In summary, we have developed new, efficient, and total syntheses of the naturally occurring AAs I–IV together with a series of metabolites and AA-analogues. These include the proposed penultimate AA metabolites involved in DNA adduct formation, namely, the *N*-sulfonyloxy AA-lactams derived from AA I and AA II and the corresponding putative metabolites, the *N*-acetoxy AA-lactams, together with two AAs having a 3-aminopropyloxy linker at the 7-position. The latter are precursors for the preparation of derivatives containing a biological marker, either biotin or a fluorescent probe. The total synthesis of these substances in quantity has not only allowed a broad array of biological studies to be carried out but also has provided the chemical basis for the site-specific incorporation of AA metabolites into oligomeric DNA of any designated sequence (unpublished material).

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■ ASSOCIATED CONTENT

Supporting Information

Details of the synthetic methods; NMR and MS data for the characterization of each intermediate or final product in the synthesis of AA or related compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AA, aristolochic acid; AAN, aristolochic acid nephropathy; BEN, Balkan endemic nephropathy; Boc, *tert*-butoxycarbonyl; BPin, boron pinacolate; $B(Pin)_2$, bis-pinacolato diboron; BuLi, butyl lithium; CHN, Chinese herbs nephropathy; DHP, dihydropyran; DIBAL, di-isobutylaluminum hydride; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; NAT, *N*-acetyl transferase; NPA, 8-nitro-1-naphthoic acid; NQO1, NAD(P)H dehydrogenase, quinone 1; Pyr, pyridine; SULT1B1, sulfotransferase 1B1; TBDMS, *tert*-butyldimethylsilyl; Tf_2O, triflic anhydride; THP, tetrahydropyran

■ REFERENCES

(1) Heinrich, M., Chan, J., Wanke, S., Neinhuis, C., and Simmonds, M. S. J. (2009) Local uses of *Aristolochia* species and content of nephrotoxic aristolochic acid 1 and 2-A global assessment based on bibliographic sources. *J. Ethnopharmacol. 125*, 108−144.

(2) Frei, H., Würgler, F. E., Juon, H., Hall, C. B., and Graf, U. (1985) Aristolochic acid is mutagenic and recombinogenic in Drosophila genotoxicity tests. *Arch. Toxicol. 56*, 158−166.

(3) Kupchan, S. M., and Doskotch, R. W. (1962) Tumor inhibitors. I. Aristolochic acid, the active principle of *Aristolochia indica*. *J. Med. Pharm. Chem. 91*, 657−659.

(4) Jackson, L., Kofman, S., Weiss, A., and Brodovsky, H. (1964) Aristolochic acid (NSC-50413): Phase I clinical study. *Cancer Chemother. Rep. 42*, 35−37.

(5) Pezzuto, J. M., Swanson, S. M., Mar, W., Che, C. T., Cordell, G. A., and Fong, H. H. S. (1988) Evaluation of the mutagenic and cytostatic potential of aristolochic acid (3,4-methylenedioxy-8-methoxy-10-nitrophenanthrene-1-carboxylic acid) and several of its derivatives. *Mutat. Res. 206*, 447−54.

(6) Vanherweghem, J.-L., Depierreux, M., Tielemans, C., Abramowicz, D., Dratwa, M., Jadoul, M., Richard, C., Vandervelde, D., Verbeelen, D., Vanhaelen-Fastre, R., and Vanhaelen, M. (1993) Rapidly progressive interstitial renal fibrosis in young women: association with slimming regimen including Chinese herbs. *Lancet 341*, 387−391.

(7) Debelle, F. D., Vanherweghem, J.-L., and Nortier, J. L. (2008) Aristolochic acid nephropathy: A worldwide problem. *Kidney Int. 74*, 158−169.

(8) Cosyns, J.-P., Jadoul, M., Squifflet, J.-P., Van Cangh, P.-J., and van Ypersele de Strihou, C. (1994) Urothelial malignancy in nephropathy due to Chinese herbs. *Lancet 344*, 188.

(9) Cosyns, J.-P., Jadoul, M., Squifflet, J.-P., Wese, F.-X., and van Ypersele de Strihou, C. (1999) Urothelial lesions in Chinese-herb nephropathy. *Am. J. Kidney Dis. 33*, 1011−1017.

(10) Grollman, A. P., and Jelaković, B. (2007) Role of environmental toxins in endemic (Balkan) nephropathy. *J. Am. Soc. Nephrol. 18*, 2817−2823.

(11) Cosyns, J.-P., Goebbels, R.-M., Liberton, V., Schmeiser, H. H., Bieler, C. A., and Bernard, A. M. (1998) Chinese herbs nephropathy-associated slimming regimen induces tumours in the forestomach but no interstitial nephropathy in rats. *Arch. Toxicol. 72*, 738−743.

(12) Cosyns, J.-P., Jadoul, M., Squifflet, J.-P., De Plaen, J. F., Ferluga, D., and van Ypersele de Strihou, C. (1994) Chinese herbs nephropathy: a clue to Balkan endemic nephropathy? *Kidney Int. 45*, 1680−1688.

(13) Grollman, A. P. (2013) Aristolochic acid nephropathy: Harbinger of a global iatrogenic disease. *Environ. Mol. Mutagen. 54*, 1−7.

(14) Peña, J. M., Borrás, M., Ramos, J., and Montoliu, J. (1996) Rapidly progressive interstitial renal fibrosis due to a chronic intake of a herb (*Aristolochia pistolochia*) infusion. *Nephrol. Dial. Transplant. 11*, 1359−1360.

(15) Cronin, A. J., Maidment, G., Cook, T., Kite, G. C., Simmonds, M. S. J., Pusey, C. D., and Lord, G. M. (2002) Aristolochic acid as a causative factor in a case of Chinese herbal nephropathy. *Nephrol., Dial., Transplant. 17*, 524−525.

(16) Arlt, V. M., Stiborová, M., and Schmeiser, H. H. (2002) Aristolochic acid as a probable human cancer hazard in herbal remedies: a review. *Mutagenesis 17*, 265−277.

(17) Gökmen, M. R., Cosyns, J.-P., Arlt, V. M., Stiborová, M., Phillips, D. H., Schmeiser, H. H., Simmonds, M. S. J., Cook, H. T., Vanherweghem, J.-L., Nortier, J. L., and Lord, G. M. (2013) The epidemiology, diagnosis, and management of aristolochic acid nephropathy: a narrative review. *Ann. Int. Med. 158*, 469−477.

(18) Pailer, M., and Schleppnik, A. (1957) Natural plant substances with a nitro group. II. Constitution of aristolochic acid II. *Monatsh. Chem. 88*, 367−387.

(19) Pailer, M. (1960) Naturally Occurring Nitrogen Compounds, in *Fortschr. Chem. Org. Naturstoffe* (Zechmeister, L., Ed.) Vol. *18*, pp 55−82, Wein Springer Verlag.

(20) Mengs, U., Lang, W., and Poch, J.-A. (1982) The carcinogenic action of aristolochic acid in rats. *Arch. Toxicol. 51*, 107−119.

(21) Mengs, U. (1988) Tumor induction in mice following exposure to aristolochic acid. *Arch. Toxicol. 61*, 504−505.

(22) Schmeiser, H. H., Bieler, C. A., Wiessler, M., van Ypersele de Strihou, C., and Cosyns, J.-P. (1996) Detection of DNA adducts formed by aristolochic acid in renal tissue from patients with Chinese herbs nephropathy. *Cancer Res. 56*, 2025−2028.

(23) Grollman, A. P., Shibutani, S., Moriya, M., Miller, F., Wu, L., Moll, U., Suzuki, N., Fernandes, A., Rosenquist, T., Medverec, Z., Jakovina, K., Brdar, B., Slade, N., Turesky, R. J., Goodenough, A. K., Rieger, R., Vukelić, M., and Jelaković, B. (2007) Aristolochic acid and the etiology of endemic (Balkan) nephropathy. *Proc. Natl. Acad. Sci. U.S.A. 104*, 12129−12134.

(24) Pfau, W., Schmeiser, H. H., and Wiessler, M. (1990) Aristolochic acid binds covalently to the exocyclic amino group of purine nucleotides in DNA. *Carcinogenesis 11*, 313−319.

(25) Krumbiegel, G., Hallensleben, J., Mennicke, W. H., Rittmann, N., and Roth, H. J. (1987) Studies on the metabolism of aristolochic acids I and II. *Xenobiotica 17*, 981−991.

(26) Turesky, R. J., and Le Marchand, L. (2011) Metabolism and biomarkers of heterocyclic aromatic amines in molecular epidemiology studies: lessons learned from aromatic amines. *Chem. Res. Toxicol. 24*, 1169−1214.

(27) Stiborová, M., Frei, E., Sopko, B., Sopková, K., Marková, V., Laňková, M., Kumstýřová, T., Wiessler, M., and Schmeiser, H. H. (2003) Human cytosolic enzymes involved in the metabolic activation

of carcinogenic aristolochic acid: evidence for reductive activation by human NAD(P)H:quinone oxidoreductase. *Carcinogenesis 24*, 1695−1703.
(28) Johnson, F. (1968) Allylic strain in six-membered rings. *Chem. Rev. 68*, 375−413.
(29) Rule, H. G., and Barnett, A. J. G. (1932) Reactivity of peri-substituted naphthalenes. I. Displacement of the nitro group in 8-nitro-1-naphthoic acid by thionyl halides to form 8-chloro- and 8-bromonaphthoic acids. *J. Chem. Soc.*, 175−179.
(30) Kupchan, S. M., and Wormser, H. C. (1965) Tumor inhibitors. X. Photochemical synthesis of phenanthrenes. Synthesis of aristolochic acid and related compounds. *J. Org. Chem. 30*, 3792−3800.
(31) Pailer, M., Berner, H., and Makleit, S. (1967) Plant substances with a nitro group. VII. Synthesis of aristolochic acid-IV methyl ester. *Monatsh. Chem. 98*, 1603−1612.
(32) Estévez, J. C., Estévez, R. J., Guitián, E., Villaverde, M. C., and Castedo, L. (1989) Intramolecular aryne cycloaddition approach to aristolactams. *Tetrahedron Lett. 30*, 5785−5786.
(33) Couture, A., Deniau, E., Grandclaudon, P., and Hoarau, C. (1998) Total syntheses of taliscanine, velutinam, and enterocarpam II. *J. Org. Chem. 63*, 3128−3132.
(34) Rys, V., Couture, A., Deniau, E., and Grandclaudon, P. (2003) A short total synthesis of the alkaloids piperolactam C, goniopedaline, and stigmalactam. *Eur. J. Org. Chem.*, 1231−1237.
(35) Kim, Y. H., Lee, H., Kim, Y. J., Kim, B. T., and Heo, J.-N. (2008) Direct one-pot synthesis of phenanthrenes via *Suzuki-Miyaura* coupling/aldol condensation cascade reaction. *J. Org. Chem. 73*, 495−501.
(36) Kim, J. K., Kim, Y. H., Nam, H. T., Kim, B. T., and Heo, J.-N. (2008) Total synthesis of aristolactams via a one-pot *Suzuki-Miyaura* coupling/aldol condensation cascade reaction. *Org. Lett. 10*, 3543−3546.
(37) Rigby, J. H., Cavezza, A., and Heeg, M. J. (1998) Total synthesis of (±)-tazettine. *J. Am. Chem. Soc. 120*, 3664−3670.
(38) Ziegler, F. E., and Fowler, K. W. (1976) Substitution reactions of specifically ortho-metalated piperonal cyclohexylimine. *J. Org. Chem. 41*, 1564−1566.
(39) Olah, G. A., Ramaiah, P., Lee, C. S., and Prakash, G. K. S. (1992) Synthetic methods and reactions. 175. Convenient oxidation of oximes to nitro compounds with sodium perborate in glacial acetic acid. *Synlett*, 337−339.
(40) Emmons, W. D., and Pagano, A. S. (1955) Peroxytrifluoroacetic acid. VI. The oxidation of oximes to nitroparaffins. *J. Am. Chem. Soc. 77*, 4557−4559.
(41) Takamoto, T., Ikeda, Y., Tachimori, Y., Seta, A., and Sudoh, R. (1978) Novel synthesis of γ-hydroxy-α-nitro-olefins. *J. Chem. Soc., Chem. Commun.*, 350−351.
(42) Sakakibara, T., Ikeda, Y., and Sudoh, R. (1982) Preparation of α-nitro olefins from α-halo ketoximes. *Bull. Soc. Chim. Jpn. 55*, 635−636.
(43) Ballini, R., Marcantoni, E., and Petrini, M. (1992) Synthesis of functionalized nitroalkanes by oxidation of oximes with urea-hydrogen peroxide complex and trifluoroacetic anhydride. *Tetrahedron Lett. 33*, 4835−4838.
(44) Bose, D. S., and Vanajatha, G. (1998) A versatile method for the conversion of oximes to nitroalkanes. *Syn. Comm. 28*, 4531−4535.
(45) Campestrini, S., Di Furia, F., Rossi, P., Torboli, A., and Valle, G. (1993) Comparison of the relative efficiency of peroxomolybdenum complexes as oxidants of the alcoholic function. *J. Mol. Catal. 83*, 95−105.
(46) Ballistreri, F. P., Barbuzzi, E., Tomaselli, G. A., and Toscano, R. M. (1996) Useful oxidation procedure of oximes to nitro compounds with benz-Mo in acetonitrile. *Synlett*, 1093−1094.
(47) Choshi, T., Kumemura, T., Nobuhiro, J., and Hibino, S. (2008) Novel synthesis of the 2-azaanthraquinone alkaloid, scorpinone, based on two microwave-assisted pericyclic reactions. *Tetrahedron Lett. 49*, 3725−3728.
(48) Bajwa, N., and Jennings, M. P. (2006) Efficient and selective reduction protocols of the 2,2-dimethyl-1,3-benzodioxan-4-one functional group to readily provide both substituted salicylaldehydes and 2-hydroxybenzyl alcohols. *J. Org. Chem. 71*, 3646−3649.
(49) Lajide, L., Escoubas, P., and Mizutani, J. (1993) Antifeedant activity of metabolites of Aristolochia albida against the tobacco cutworm *Spodoptera litura*. *J. Agric. Food Chem. 41*, 669−673.
(50) Jiang, X., Garcia-Fortanet, J., and De Brabander, J. K. (2005) Synthesis and complete stereochemical assignment of psymberin/irciniastatin A. *J. Am. Chem. Soc. 127*, 11254−12255.
(51) Mix, D. B., Guinaudeau, H., and Shamma, M. (1982) The aristolochic acids and aristolactams. *J. Nat. Prod. 45*, 657−666.
(52) Sidorenko, V. S., Attaluri, S., Zaitseva, I., Iden, C. R., Dickman, K., Johnson, F., and Grollman, A. P. (2014) Bioactivation of the human carcinogen aristolochic acid. *Carcinogenesis*, DOI: 10.1093/carcin/bgu095.