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TRUNCATED ASPIDOSPERMA ALKALOID-LIKE SCAFFOLDS: UNIQUE STRUCTURES FOR THE DISCOVERY OF NEW, BIOACTIVE COMPOUNDS

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Abstract – Exploration into the dienophilicity of indole in inverse electron demand Diels-Alder reactions has led to the development of the triazatetracyclo[7.7.0.0^{1,13}.0^{2,7}]hexadeca-2,4,6,10,12-pentaene and related scaffolds for diversification in the search for new, biologically active compounds. The libraries constructed from these core structures have lead to the discovery of new anti-viral and anti-malarial compounds.

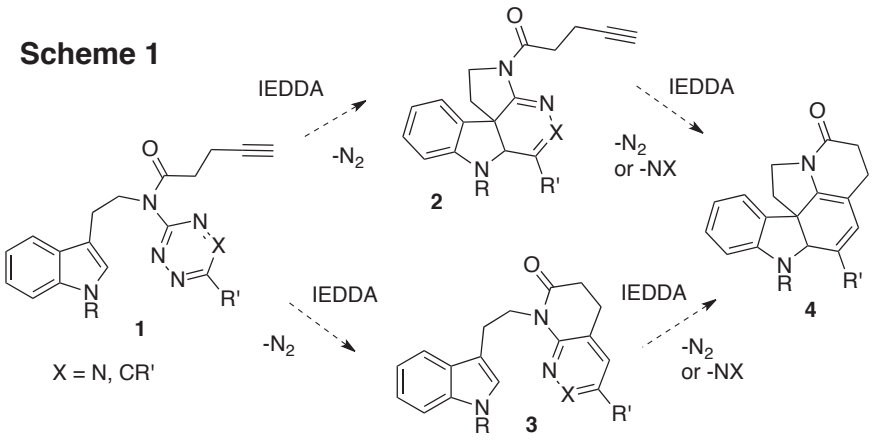
INTRODUCTION

We have long been interested in the inverse electron demand Diels-Alder (IEDDA) chemistry of heteroaromatic compounds with latent enamine functionalities serving as dienophiles. Within this general

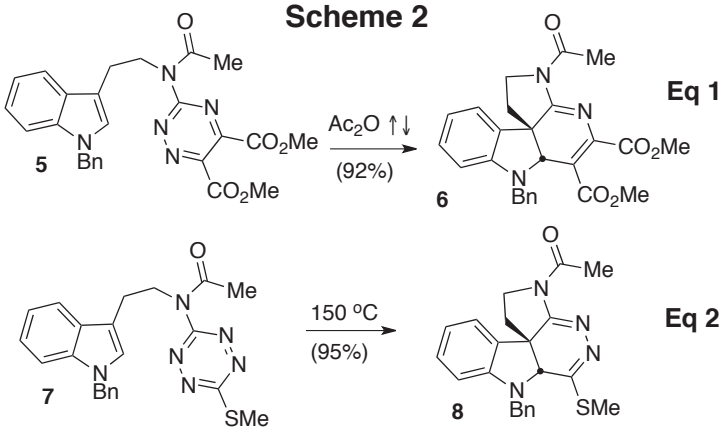
theme, we have focused primarily on the dienophilicity of indole,[1] and to a lesser extent pyrrole[2] and imidazole,[3] in reactions with electron deficient heteroaromatic azadienes, and applied this chemistry to the synthesis of new heterocycles. Indeed, these are areas of research which Professor Al Padwa has greatly influenced as a prolific leader with his ground-breaking work. Most notable in this effort is his work with the cycloadditions of indole with electron deficient dienes and dipoles applied to the synthesis of a large number of alkaloids and alkaloidal analogues.[4]

Quite some time ago we began an investigation into the use of a sequence of inverse electron demand Diels-Alder reactions between 1,2,4,5-tetrazines (**1**, X = N) tethered to tryptamine or tryptophan as a route to access the *Aspidosperma* alkaloidal skeleton **4** (Scheme 1).[1b,5] Simultaneously, an analogous approach with tethered 1,2,4-triazines (**1**, X = CR') was also developed. This work began with an examination into the dienophilicity of the indole subunits of tryptamine and tryptophan with tethered tetrazines and triazines in intramolecular IEDDA reactions, which proved to be very successful, exemplified by the reactions in Scheme 2. Linking the heterocycle to the tryptamine or tryptophan amino group was easily accomplished by simple S_NAr chemistry with displacement of an appropriate leaving group (OMe, SMe, or Cl).[1b] In the course of this work, it was discovered that in order for the reaction to proceed, the tethering nitrogen linking the tetrazine to the tryptamine must be acylated to reduce electron donation from the amine lone pair into the heteroaromatic diene. The extent of this electron donation in non-acylated tethered triazines was readily apparent from the ^{1}H NMR spectra of the tethered 1,2,4-triazines which displayed slowly interconverting rotamers reminiscent of rotamers frequently observed with amides (Figure 1). Indeed, the ~0.8 ppm downfield shift of the H-9 methylene protons of tryptamine upon tethering of the triazines was reminiscent of acylation shifts upon amide formation.[6] Furthermore, we speculated that in the case of tethered triazines, the IEDDA reaction required a second acylation of a triazine nitrogen since

Scheme 1



Scheme 2



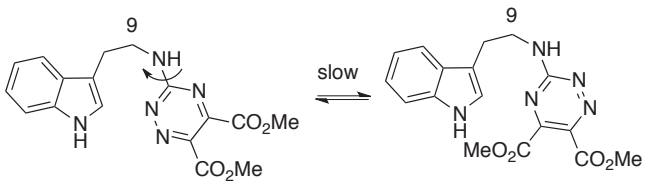
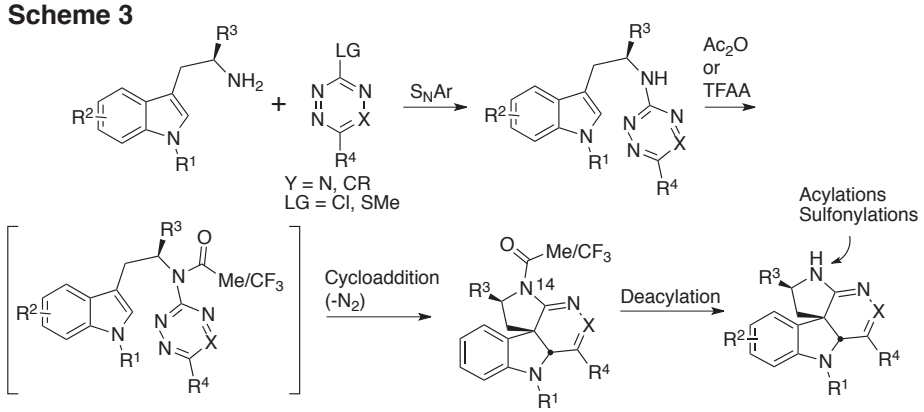


Figure 1. Slowly interconverting rotamers of tryptamine-tethered triazine

N-acetyl tethered tryptamine/triazine pair **5** did not undergo a cycloaddition upon heating to 232 ºC, though the reaction proceeded smoothly in refluxing Ac_2O (bp 140 ºC, Scheme 2, Eq 1). *N*-Acylated tethered tetrazines such as **7** did participate in the thermally promoted cycloadditions in the absence of in situ acylation (Scheme 2, Eq 2).

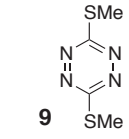
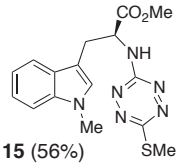
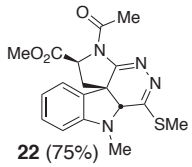
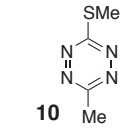
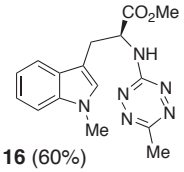
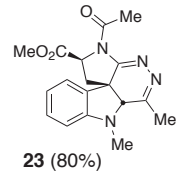
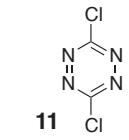
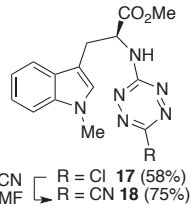
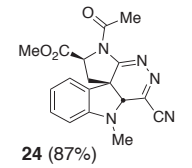
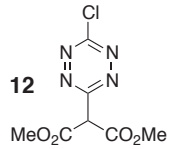
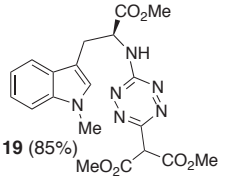
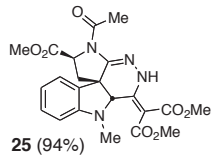
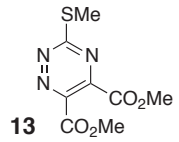
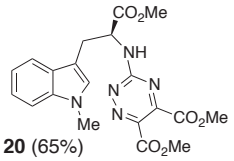
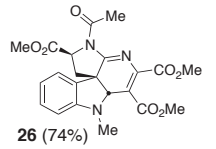
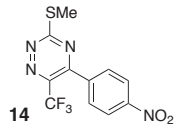
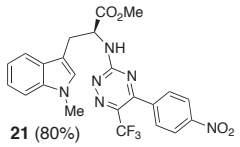
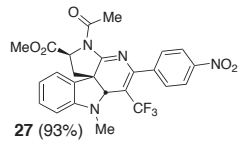
The intramolecular cycloadditions of various tethered tetrazines and triazines could be accomplished by refluxing in acetic anhydride or in *o*-dichlorobenzene in the presence of trifluoroacetic anhydride (TFAA). With tryptophan derivatives, only single diastereomers were obtained, indicating that the stereogenic center of this amino acid is able to control the facial approach of the electron-rich indole to the electron deficient heteroaromatic azadiene (Scheme 3).[1b] Acylation of the tethering nitrogen presumably occurs prior to the cycloaddition. In acetic anhydride, the cycloadducts were isolated as the *N*-14 acetyl derivatives (Table 1). With TFAA, the trifluoroacetate group was cleaved during chromatography on SiO_2, thereby leaving this position poised for diversification. All of the tetrazines tethered to either tryptamine or tryptophan participated in IEDDA reactions under acetylation conditions, producing the desired cycloadducts in good to excellent yields. With electron-withdrawing substituents at C-6, the cycloadditions of tethered tetrazines could be accomplished under relatively mild conditions, proceeding at temperatures as low as rt to 40 ºC (Table 1, Items 3 and 4) Less electron deficient tetrazines required heating to 180 ºC (Table 1, Items 1 and 2). Chlorotetrazine **17** also underwent a cycloaddition upon heating to 40 ºC, but the cycloadduct proved to be unstable. Nonetheless, **17** proved to be an excellent precursor to tetrazinylnitrile **18**, which also participated in the cycloaddition, producing **24** (Table 1, Item 3). In general, the tethered triazines required two electron withdrawing groups at the triazinyl C-5 and C-6 positions, (Table 1, Items 5 and 6) as well as the higher temperatures (179 - 180 ºC) in order to accomplish the cycloaddition.

Scheme 3

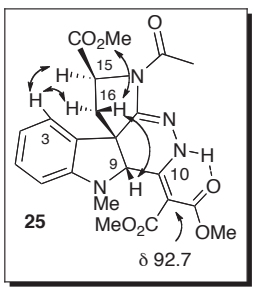


Interestingly, the C-rings of tetrazine cycloadducts **8**, **22**, **23**, and **24** were in the 1,2-dihydro tautomeric states, as evidenced by the C-9 methine singlet in the δ 3.80 – 4.35 range, depending on the C-10 substituent, with the corresponding carbon at δ 67.0 – 70.1. In cycloadduct **25** the vinylogous carbamate form of the C-ring predominated exclusively (Figure 2). This tautomer was initially suggested by the low field singlet at δ 11.48 (NH) in the ^{1}H NMR spectrum which did not correlate with a carbon in the HSQC spectrum, and which very slowly exchanged with deuterium in D_2O-saturated $CDCl_3$ (90% exchanged after 3 days), indicating a strong intramolecular hydrogen bond.[7] This hydrogen bond is likely the cause of the predominance of the vinylogous carbamate form. Furthermore, a non-hydrogen bearing enamine-type β-carbon appeared at δ 92.7. In addition, a methine singlet was observed at δ 4.32 with the corresponding carbon at δ 67.9 assigned to H-9. An NOE between

Table 1. Tethering and Cycloadditions of Tryptophan Derivatives with Acetylation.[a]

Item	Azadiene	S_NAr Product	Cycloadduct
1[b]	 **9**	 **15** (56%)	 **22** (75%)
2[b]	 **10**	 **16** (60%)	 **23** (80%)
3[c]	 **11**	 KCN DMF → R = Cl **17** (58%), R = CN **18** (75%)	 **24** (87%)
4[d]	 **12**	 **19** (85%)	 **25** (94%)
5[b]	 **13**	 **20** (65%)	 **26** (74%)
6[b]	 **14**	 **21** (80%)	 **27** (93%)

(a) Isolated yields. (b) Cycloaddition conditions: Reflux in *o*-dichlorobenzene (179-180 °C), 4 h. (c) Cycloaddition conditions: rt, CH_2Cl_2 1 h, then reflux (39-40 °C) 1 h. (d) Cycloaddition conditions: Reflux in CH_2Cl_2 2 h.



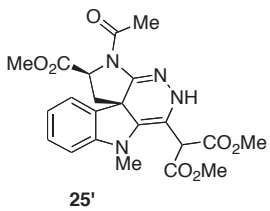


Figure 2. NOE's supporting tautomeric form **25**, ruling out **25'**

this ring fusion methine proton and H-16b not only supported the *cis* B,C-ring fusion, but also ruled out the 1,4-dihydropyridazine tautomer **25'**. As with other cycloadducts, NOE's from H3 to both H15 and H16a allowed assignment of the facial selectivity in the

cycloaddition, and hence the absolute stereochemistry given the enantiopurity of the starting tryptophan. As noted above, the triazines were much less reactive than the tetrazines. Only those tethered triazines with two electron-withdrawing groups at the C-5 and C-6 positions participated in the cycloadditions. Those triazines which failed to undergo the intramolecular Diels-Alder reactions are shown in Figure 3. Emphasizing the importance of two electron withdrawing substituents was the failure of triazine **31**, with a single ester substituent, to react with either tryptophan or tryptamine. Triazine **14** (Table 1, Item 6) represents a previously unreported heterocycle, which was surprisingly made with complete regioselectivity by the condensation of dione **32** with *S*-methylisothiosemicarbazide (**Scheme 4**). The regioselectivity of this cyclocondensation was confirmed by ^{13}C-^{19}F couplings observed in the ^{13}C-NMR spectrum of the cycloadduct **27** (*Inset*), notably ^{2}J = 29.8 Hz to C-10 (δ 109.7) and ^{3}J = 3.7 Hz to C-11 (δ 140.5). In addition, ^{5}J = 2.3 Hz to the *N*-methyl carbon (δ 37.7) was also seen, with the Fermi contact presumably propagated by fluorine interaction with spatially close *N*-methyl protons.[8]

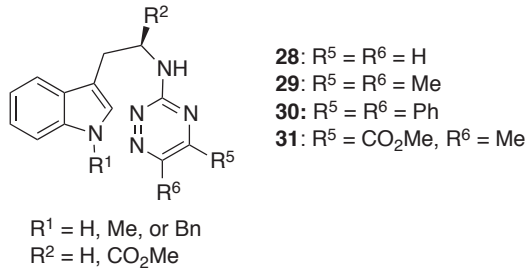
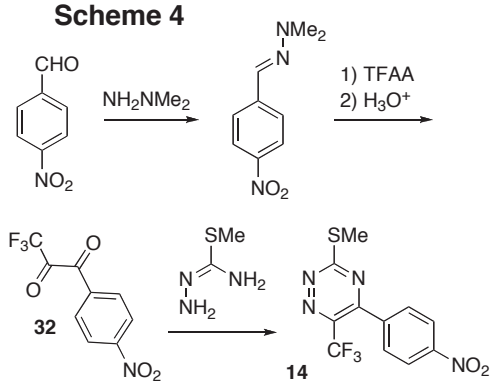
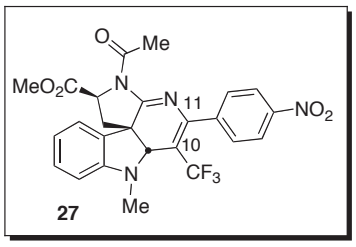


Figure 3. Triazines which failed to produce cycloadducts

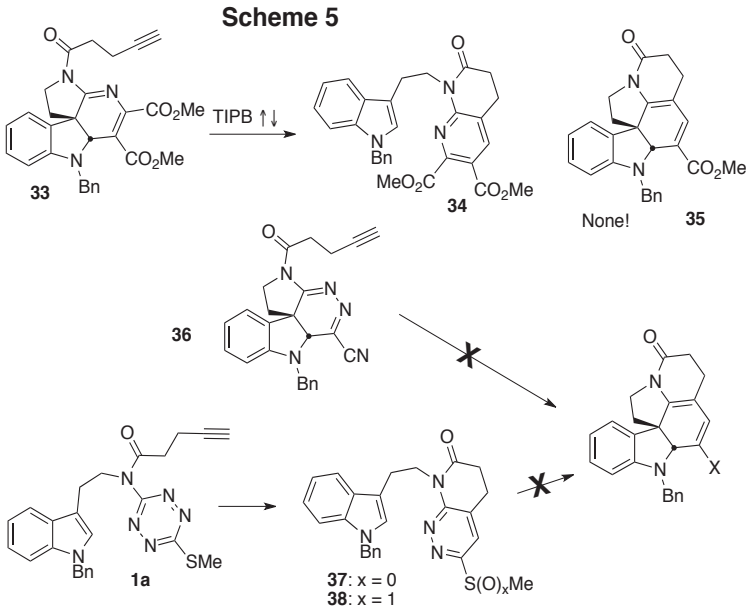
Scheme 4





Unfortunately, we were never able to affect a second cycloaddition in the designed sequence to **4** (cf. Scheme 1). Triazine cycloadduct **33**, produced by the cycloaddition in TFAA followed by acylation with pentynoyl anhydride,[1b] underwent a second cycloaddition as hoped, but the cycloreversion produced the pyridine subunit **34** rather than the desired pentacyclic core **35**, which would have formed by the release of methyl cyanformate (Scheme 5). The analogous pentynoylated tethered cycloadduct **36**, failed to participate in further cycloadditions. The alkyne in tethered tetrazine **1a**, proved to be more dienophilic than the indole subunit, producing pyridazine **37** (x = 0) in near quantitative yield. Neither this thiomethyl pyridazine cycloadduct, nor the sulfoxide **38** (x = 1), produced by *m*-CPBA oxidation of **37**, underwent a cycloaddition under the many conditions investigated.

Ultimately, Dale Boger cleverly showed, by constructing a 1,3,4-oxadiazole heteroaromatic diene system onto tryptamine, that the desired Aspidosperma scaffolds could be obtained following the tandem cycloaddition strategy.[9] Boger's group has subsequently applied this chemistry to the synthesis of a number of Aspidosperma and Vinca alkaloids, including minovine,[9] fendleridine,[10] vindorosine,[11] vindoline[12] and their analogues.[13]



Our efforts in the cycloaddition chemistry of tryptamine and tryptophan went into hibernation after the publication in 2000.[1b] However, the rise of diversity oriented synthesis (DOS)[14] as a means to build upon complex, non-natural scaffolds led us to consider the development of the 8,12,14-triazatetracyclo[7.7.0.0^{1,13}.0^{2,7}]hexadeca-2,4,6,10,12-pentaene (**39**) and 8,11,12,14-tetraazatetracyclo[7.7.0.0^{1,13}.0^{2,7}]hexadeca-2,4,6,10,12-pentaene (**40**) cycloadduct cores (**Figure 4**) as scaffolds for library synthesis. The rapid assembly of complex molecular libraries in DOS has become an important approach to discovering novel chemotypes as new pharmacological tools. Libraries with scaffolds that structurally mimic natural products can provide to new leads with unique pharmacological properties.[15] Several comparatively simple indole-based libraries have been reported,[16] as have more complex unnatural indole alkaloid-type libraries,[17] with subsequent screening revealing bioactivities worthy of further biological investigation.[18] Scaffolds **39** and **40** resemble truncated Aspidosperma alkaloids with transposed nitrogens in the C-ring, and represent ideal candidates for library synthesis and subsequent screening. These scaffolds are of relatively moderate molecular weight (350-400), with several distinct sites for elaboration (R^1 through R^6). With this in mind, the synthesis of a series of libraries based on these core structures was completed within the Center for Chemical Methodology and Library Development at Boston University (CMLD-BU),[19] an NIH funded center for reaction and novel chemotype discovery. What has emerged from this work is the recognition that these tetracyclic scaffolds exhibit biological activity against various infectious diseases differentiated by the mode of diversification.

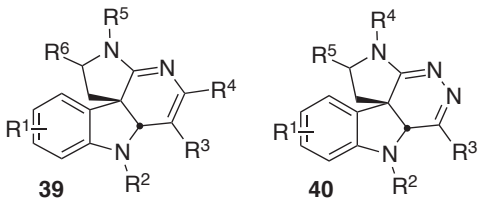
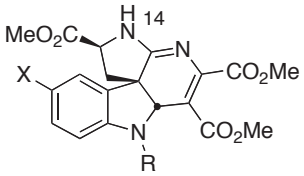


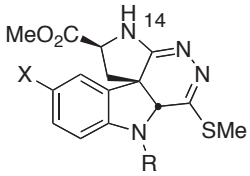
Figure 4. Aspidosperma alkaloid-inspired scaffolds **39** and **40** for library synthesis

DIVERSIFICATION OF TETRAZINE AND TRIAZINE CYCLOADDUCTS OF TRYPTOPHAN

The first generation libraries from the two scaffolds **39** and **40** employed (L)-tryptophan as the starting dienophile, resulting in diastereomerically and enantiomerically pure cycloadducts. Furthermore, (D)-tryptophan is commercially available, so the preparation of enantiomers would be straightforward. Following the strategy outlined in Scheme 3, and employing the triazine **13** and tetrazine **9** as azadienes (cf. Table 1) with TFAA as the acylating reagent to promote the cycloaddition, six triazine and six tetrazine cycloadducts, **41a**-**f** and **42a**-**f**, were prepared from the corresponding tryptophan dienophiles[20] (Figure 5). Deacylation of the trifluoroacetate group occurred during flash chromatography on silica gel, rendering the *N*-14 position available for diversification. These twelve scaffolds were prepared on 2 g scales.



41a: R = H = H
41b: R = Me, X = H
41c: R = Bn, X = H
41d: R = $CH_2CH{=}CH_2$, X = H
41e: R = $-CH_2-{\equiv}-Me$, X = H
41f: R = Me, X = OMe

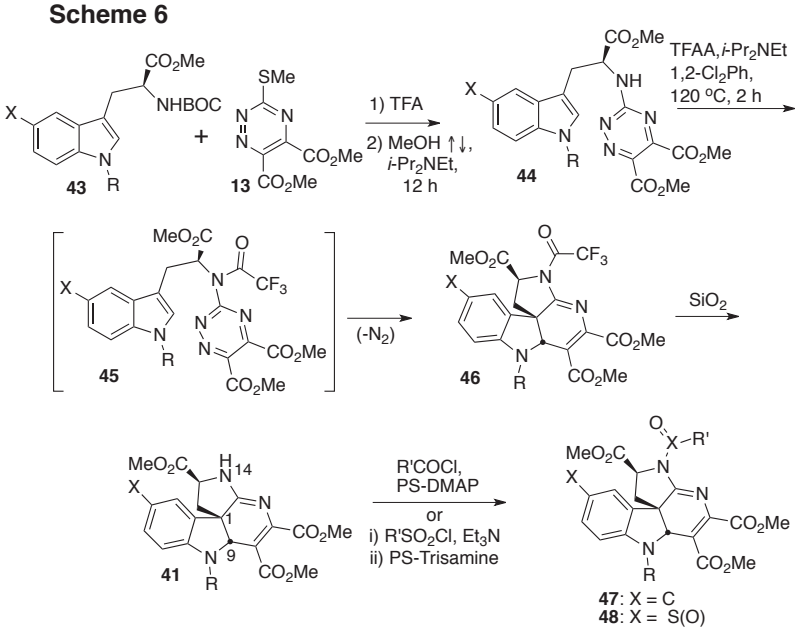


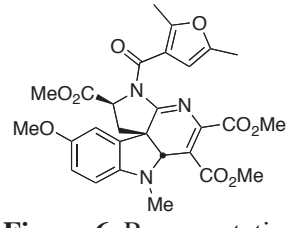
42a: R = H = H
42b: R = Me, X = H
42c: R = Bn, X = H
42d: R = $CH_2CH{=}CH_2$, X = H
42e: R = $-CH_2-{\equiv}-Me$, X = H
42f: R = Me, X = OMe

Figure 5. Triazine- and tetrazine-derived scaffolds for diversification

The scaffolds were subsequently diversified through acylations and sulfonylations with seventeen commercially available acid chlorides and ten sulfonyl chlorides.[20] As illustrated for the triazine-derived cycloadducts (Scheme 6), the acylations used PS-DMAP[21] as a base promoter, while the sulfonylations utilized Et_3N, with subsequent treatment with PS-trisamine[22] to scavenge excess sulfonyl chloride. No competing acylation or sulfonylation of the unprotected indoline nitrogen was observed in the diversification of scaffolds **41a** and **42a** (R^1 = H). Final purification was achieved through mass directed LC-MS. Representative members of the library prepared are shown in Figure 6. The formation of ureas **49** from scaffolds **41** was also investigated (Scheme 7). While the ureas could be isolated in good yields, they proved unstable to storage, undergoing a deacylation,

Scheme 6





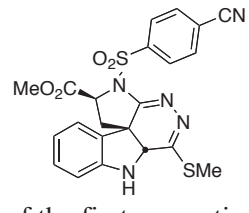
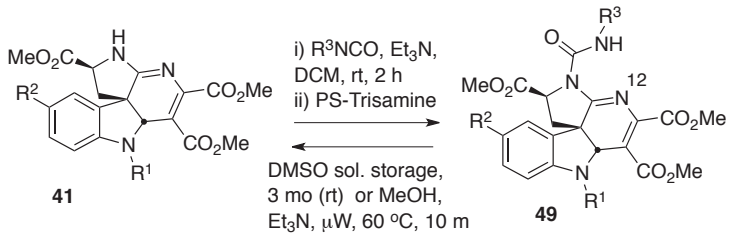


Figure 6. Representatives of the first generation of truncated Apsidosperma alkaloid-like library

Scheme 7



regenerating the original scaffold **41** and the isocyanate. This instability is perhaps not surprising given the potential of the dihydropyridine ring *N*-12 to function as an intramolecular base catalyst for urea cleavage.[23] Base treatment of **49** also resulted in complete deacylation with isocyanate release and recovery of **41**. Consequently, these ureas were not further considered for inclusion in a library.

The library members from both scaffolds **41** and **42** were stable upon storage on the bench-top in the solid state. While the triazine adducts were also stable in a variety of solvents including DMSO, the tetrazine analogues slowly formed adducts with DMSO upon storage in that solvent, as seen by UPLC. The DMSO adduct presumably forms at C-13 position of the tetrazine cycloadducts, a position sensitive to addition of other nucleophiles such as primary amines.[24] No such adducts were observed with the triazine cycloadducts, wherein the C-13 is considerably less electrophilic. Given the reactivity of tetrazine-derived library members from scaffolds **42** in DMSO, these compounds were not submitted for biological evaluation.

The 132-membered library prepared from the triazine cycloadducts were screened for biological activity in a variety of assays as part of the CMLD-BU small molecule collection. Three different assays showed "Hits" from this unnatural alkaloid library, as defined by the collaborators performing the assay (Figure 7). These assays were: (1) inhibition of the dimerization of the capsid protein of the hepatitis C virus (HCV), known as "core";[25] (2) proliferation inhibition of several strains of the malaria-inducing *Plasmodium falciparum*;[26] and (3)

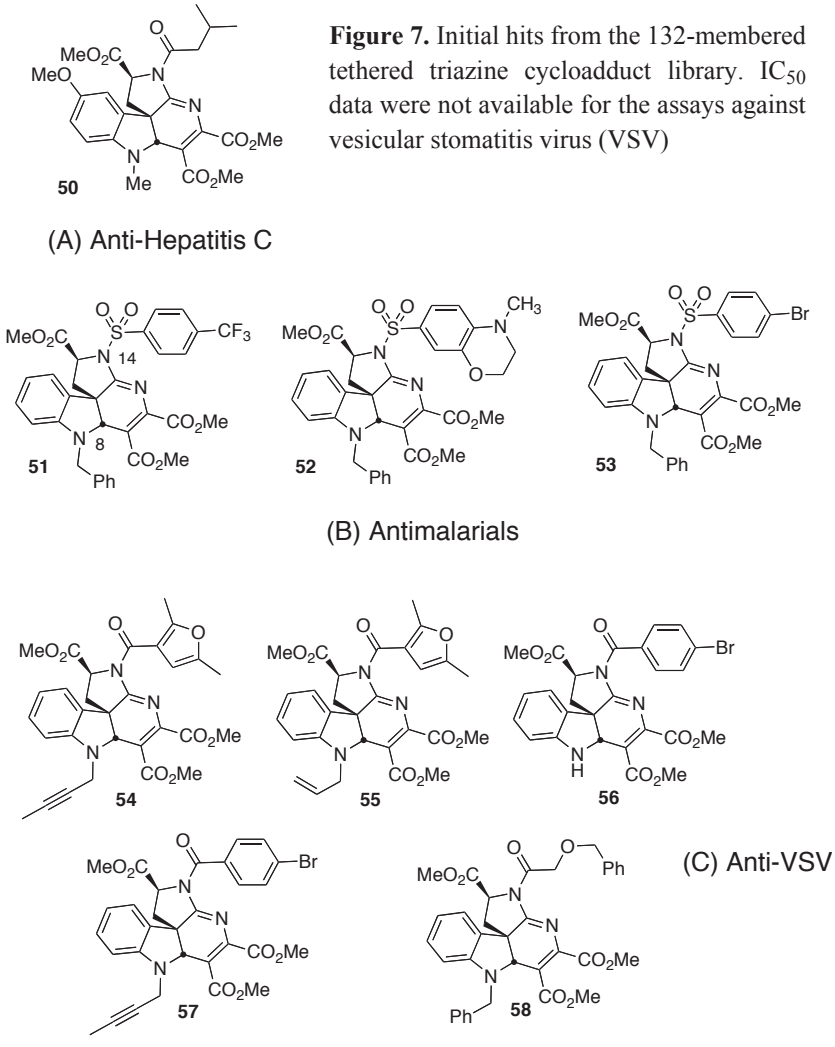


Figure 7. Initial hits from the 132-membered tethered triazine cycloadduct library. IC_{50} data were not available for the assays against vesicular stomatitis virus (VSV)

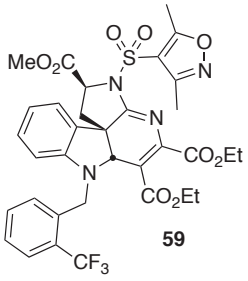
inhibition of replication of the vesicular stomatitis virus (VSV), a negative strand RNA virus.[27] All hits were validated by resubmission of fresh samples to the assay. The active compounds were specific to the particular assay; thus, there was no cross-over of hits from one assay to the other at the tested concentrations.

THE BIOASSAYS AND THE HITS

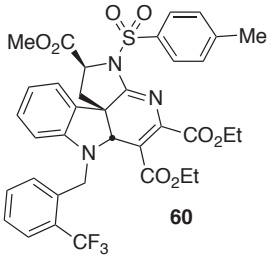
Hepatitis C, a major cause of liver disease, infects over 130 million people worldwide. The causative agent is a positive sense, single strand RNA virus, which was first reported in 1989.[28] Core is one of ten proteins encoded by the HCV RNA genome, and is required for viral assembly.[29] Inhibition of core dimerization has been demonstrated to prevent HCV proliferation in infected liver cells,[25,30] and as such core represents an attractive non-enzymatic target for the development of potential HCV treatment. Our collaborator in this work was Professor Donny Strosberg and co-workers of the Scripps Research Institute who have developed the core dimerization inhibition assay.[25] Compound **50** from the library showed activity (IC_{50} 5.7 – 9.3 μM) in this assay (Figure 7A). Given the single hit with an IC_{50} < 10 μM, it was difficult to develop an SAR assessment, other than the fact that only *N*-14 acylated analogues showed any activity at all, *N*-sulfonylated analogues within this library were invariably inactive.

Malaria is a pernicious disease that has ravaged human civilization, and continues to devastate underdeveloped nations where the impact can be difficult to fully define.[31] Indeed, this disease is, at least in part, responsible for many regions of the world continued underdeveloped state as it imposes an enormous societal burden on populations economically ill-equipped to deal with its consequences.[32] Strains of the malaria-inducing protozoan *Plasmodium falciparum* resistant to the traditional treatment with Cinchona alkaloids and their derivatives have emerged. James Inglese and his colleagues at the NIH's Chemical Genomics Center and NIAID have established a quantitative high-throughput screening (qHTS) platform examining five strains of *P. falciparum* in an effort to discover new leads against malaria, and also identify differential activities among the strains, thereby enabling determination of the genetic locus mediating the activity when patterns of inheritance are mapped from recombinant progeny. Three compounds from this library (**51** – **53**) showed activity (EC_{50}) against several strains of *P. falciparum* in the single digit μM range or below (Figure 7B).[33]

Preliminary SAR analysis suggested that, in contrast to the anti-HCV compounds, only *N*-14 sulfonylated library members were active against the malarial strains. Moreover, only analogues derived from the *N*-8 benzyl scaffold showed activity at the sub-micromolar level. Following this observation, 40 additional analogues were synthesized using the inverse electron demand Diels-Alder strategy, and screened for anti-malarial activity, varying the *N*-8 benzyl substituents and the *N*-14 sulfonamide groups.[33] Those compounds with *o*-substituted benzyl substituents at *N*-8 were found to be the most active, with the most



59



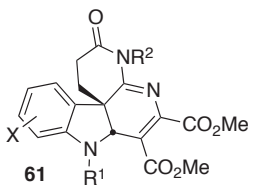
60

effective substituent being the trifluoromethyl group. Two new cycloadducts (**59** and **60**) were discovered form this focused library with single digit micromolar or sub-micromolar activity against five strains of *P. falciparum* (IC_{50} 0.6 – 0.7 μM for **59**, 0.6 – 0.8 M for **60**, depending on the strain).[33]

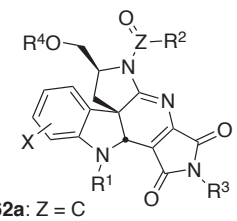
Different library members were also identified as hits in a screen for antiviral compounds that target vesicular stomatitis virus (VSV). Vesicular stomatitis virus is a veterinary virus used to determine compounds with potential activity against human pathogenic viruses. The screen itself was a cell-based assay for the inhibition of viral replication, which was developed by Professor John Connor of the Boston University School of Medicine, who has previously used VSV to identify broad spectrum antiviral agents.[27,34] The assay design was a "dual screen" that simultaneously identified compounds that (1) inhibited the replication of virus, and (2) were not cytotoxic to cells.[35] In this assay, compounds that protected cells from virus-mediated apoptosis and did not cause cell death were deemed active. Five compounds, **54** – **58**, from the library were found to be active with EC_{50}'s in the 12.5 – 50 μM range (Figure 7C). As with the HCV activity, all of these compounds were derived from N-14 acylation. Furthermore, none of these anti-VSV hits were active against HCV core dimerization.

SECOND GENERATION LIBRARIES

With these hits confirmed, two additional libraries were prepared, focusing on the HCV and anti-malarial activities. One library expanded the D-ring with the formation of a δ–lactam ring (**61**), initially focusing on two points of diversion R^1 and R^2. The second library **62** was prepared by converting the two triazine-derived ester groups of **47** and **48** into a cyclic imide.



61

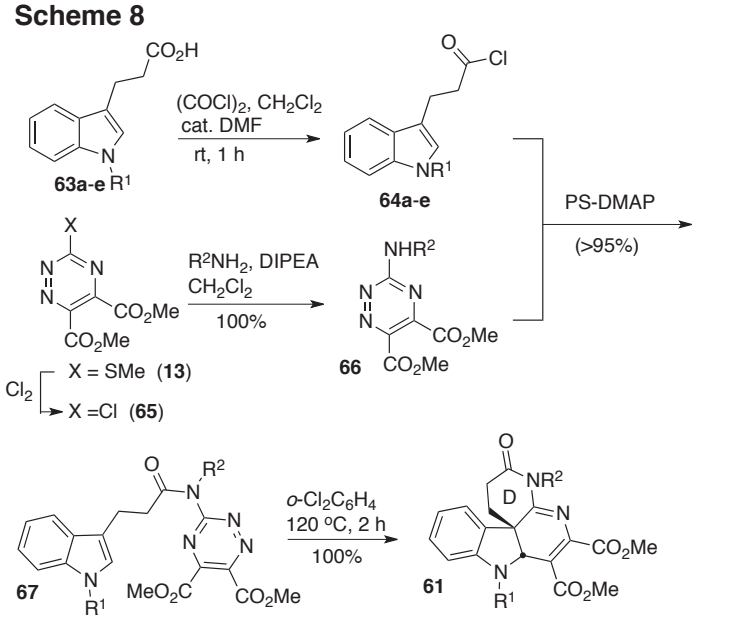


62a: Z = C
62b: Z = S(O)

Construction of the δ-lactam library **61** began with the preparation of *N*-alkylated-3-indolylpropionic acid derivatives **63** (Scheme 8).[20] *N*-Methylation of indolylpropionic acid to **63a** proceeded through *N*,*O*-dimethylation with excess methyl iodide under basic conditions, followed by basic hydrolysis of the methyl ester. Other *N*-alkyl derivatives **63b**-**e** were prepared from the methyl ester of indolylpropionic acid by *N*-alkylation with the appropriate alkyl halides following KH deprotonation, then basic ester hydrolysis.

The cycloaddition precursors, the *N*-substituted indole-3-propionic acid derived amides **67**, were then prepared by acylation of various 3-aminotriazines **66** with the propionic acid derivatives. To this end, the

Scheme 8

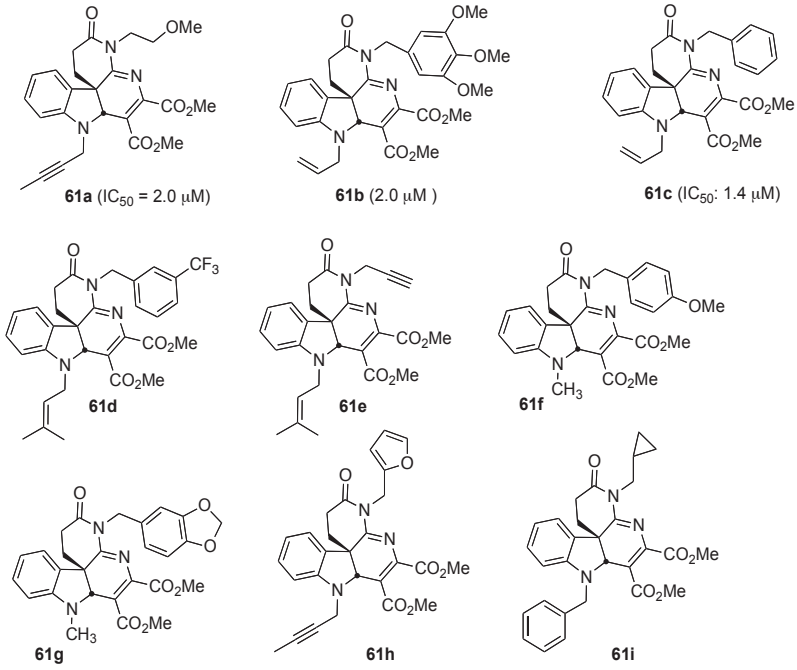


R^1 = (**a**) -CH_3, (**b**) -$CH_2CH{=}CH_2$, (**c**) -$CH_2CH{=}C(CH_3)_2$, (**d**) -CH_2Ph, (**e**)-$CH_2C{\equiv}CCH_3$

3-chloro-1,2,4-triazine **65**, prepared from thiomethyl triazine **13** upon treatment with chlorine, was reacted with various primary amines in the presence of di-*iso*--propylethylamine (DIPEA) to produce the corresponding aminotriazines **66** in near quantitative yields by S_NAr displacements. Linking the diene/dienophile pairs was accomplished by reacting **66** with *N*-substituted indole-3-propionic acid chlorides **64a**-**e** using PS-DMAP as the base promoter. Filtration through a short silica gel column removed unreacted acid chloride to give the amides **67** bearing the tethered dienophile/diene pairs in nearly quantitative yields. Heating these amides at 120 °C in *o*-dichlorobenzene for 2h resulted in the inverse electron demand intramolecular Diels-Alder reactions to give the desired cycloadducts **61**, also in near quantitative yields. The products were purified by mass-directed preparative LC-MS. Eighty-two library members, all in racemic state, were synthesized in straightforward fashion using these protocols.

Screening of this new δ-lactam library in the core dimerization inhibition assay to identify new anti-HCV candidates revealed several additional hits with single digit μM activity (IC_{50}'s, Chart 1). Three of these new active compounds (**61a–c**) were validated by resynthesis, and their biological activities were further probed. All three were approximately twice as potent as the original lead compound **50**. Furthermore, **61a** and **61c** showed no cytotoxicity against Huh7-5 hepatoma cells at concentrations up to 125 μM, and also significantly inhibited HCV viral propagation in infected hepatoma cells with EC_{50} < 1 μM.[19] None of the members of this δ-lactam library were

Chart 1. Positive Hits from δ-Lactam Library



active against malaria; these compounds were not tested against VSV.

With the improved anti-HCV activity of the δ-lactam hits, additional analogues were prepared, focusing on the tethering of a second heterocycle to the *N*-8 position. The rationale for designing new candidates with a second heterocyclic ring tethered to the initial hit(s) was the hope that this entity would find a second "hot spot" on the surface of core, resulting in stronger binding. Such an approach is not uncommon when seeking to inhibit a protein-protein interaction.[36] As recently reported,[37] two of these new compounds, **68** and **69**, showed significantly improved IC_{50}'s against core dimerization (**68**, 98 nM, **69** 341 nM), with cytotoxicity levels > 100 μM. Work is continuing to build an additional library of tethered triazoles around these results.

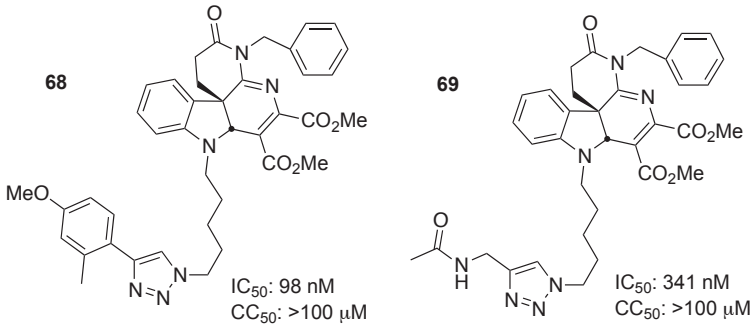
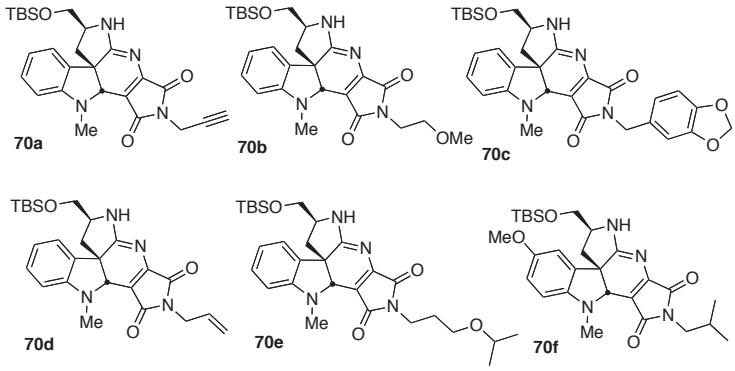
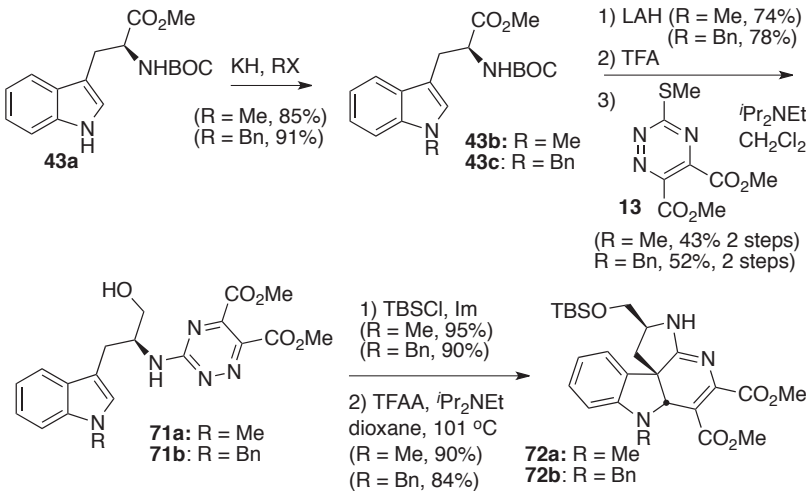


Chart 2. Cyclic Imide Sublibrary Scaffolds **70a**-**f**



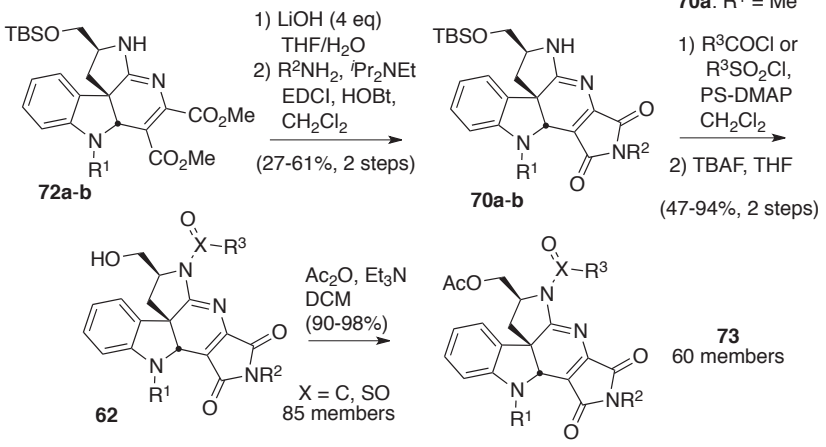
The library of cyclic imides **62** was prepared utilizing the six cycloadduct scaffolds **70a**-**f** shown in Chart 2. These scaffolds were prepared beginning with the *N*-BOC L-tryptophan methyl ester (**43a**, Scheme 9). Alkylation of the indole nitrogen following standard procedures produced **43b**-**c** (Scheme 9, RX = CH_3I, BnBr). Reduction of the methyl ester to the primary alcohol, followed by removal of the BOC group, then tethering of the triazine **13** through base promoted S_NAr displacement gave the tethered triazines **71a**-**b**. Protection of the primary alcohol as the TBS ether, then cycloaddition by refluxing in dioxane in the presence of trifluoroacetic anhydride (TFAA), yielded **72a**-**b**.

Scheme 9



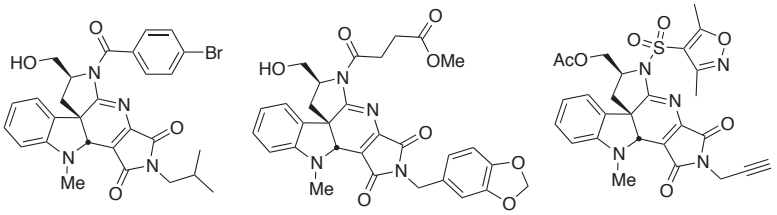
Completion of the cyclic imide scaffolds was accomplished by basic hydrolysis of

Scheme 10

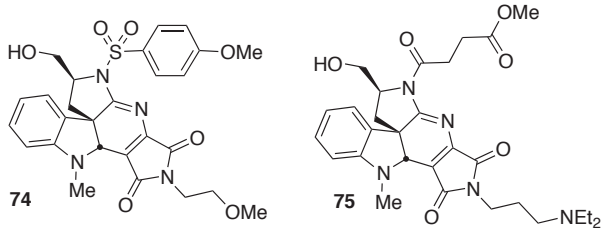


both esters, then imide formation with primary amines (Scheme 10). Diversification of scaffold **70a** by acylation and sulfonylation using ten acyl chlorides and nine sulfonyl chlorides, then desilylation with TBAF gave the first subset of 85 cyclic imide library members **62**. Sixty of these members were further acetylated at the primary alcohol as well (**73**), to produce a total number of 151 cyclic imides, which included the scaffolds **70a** - **70f**. Library members were purified by mass-directed LC-MS; representative examples are shown in Chart 3. All members of this library are single enantiomers with the chirality determined by that of the starting tryptophan.

Chart 3. Examples of the Cyclic Imide Sublibrary

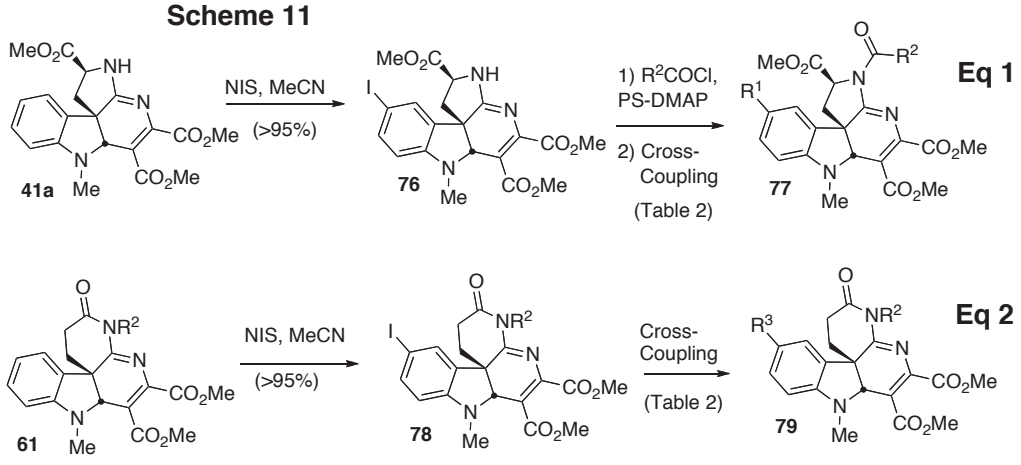


Screening of the cyclic imide library against HCV core dimerization did not reveal any members with single digit micromolar IC_{50} values, though several were identified with double digit micromolar activities. All members of the cyclic imide library were inactive against malaria. Preliminary screening of this library against VSV revealed two hits, **74** and **75**; IC_{50} values have not as yet been determine for **74** and **75**.



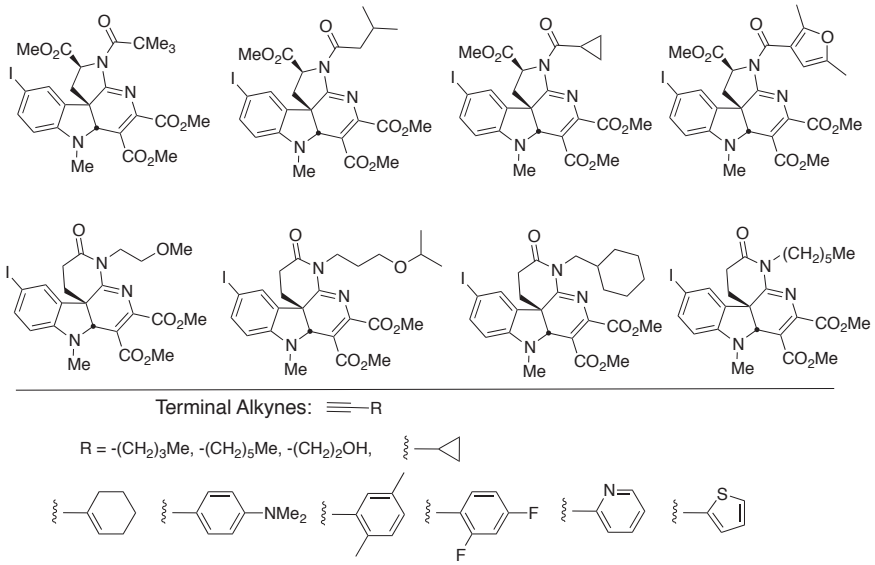
Recently, a third generation library was generated through cross-coupling chemistry employing members of the original cycloadduct library **41**, as well as the δ-lactam library **61**. Iodination of scaffold **41a** as well as select library members from the δ-lactam library **61** with NIS in acetonitrile occurred at rt (30 min for **41a**, Scheme 11, Eq 1 10 hr for **61**, Scheme 11, Eq 2).[38] In all cases, exclusive regioselectivity was observed, with iodination occurring solely at the position *para* to the original indole nitrogen. The iodinations were successful on a multigram scale.

Scheme 11



With the aryl iodides **76** and **78** in hand, various Pd-catalyzed cross-couplings were examined (Table 2). Both Suzuki[39] and Sonogashira[40] couplings proceeded smoothly under standard conditions, thereby setting the protocols for an expanded library using eight iodinated scaffolds with ten terminal alkynes in Sonogashira cross-couplings (Chart 4). An eighty-membered library has been completed, with the compounds purified by mass-directed preparative HPLC. These compounds are awaiting screening results; representative members are shown in Table 2.

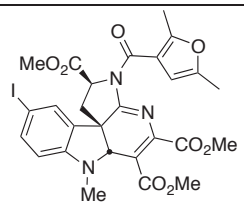
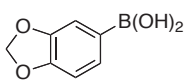
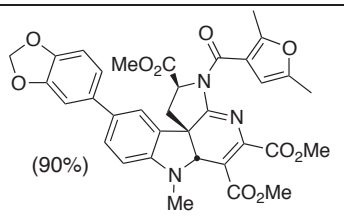
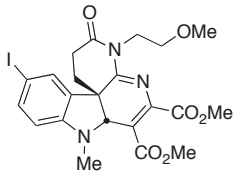
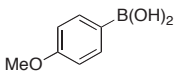
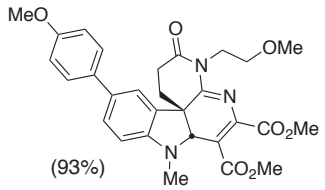
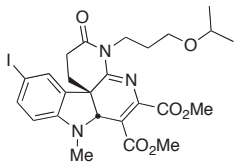
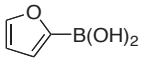
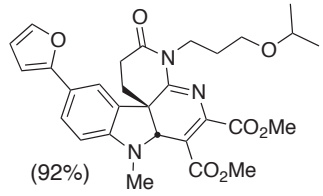
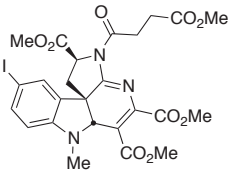
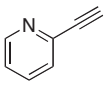
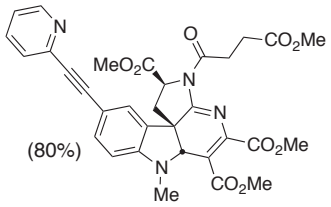
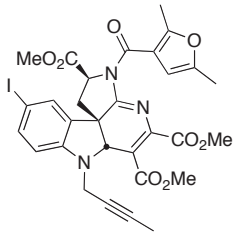
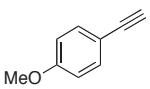
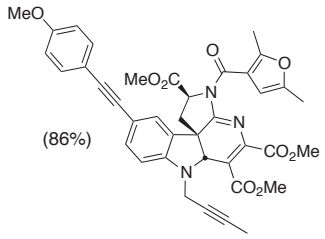
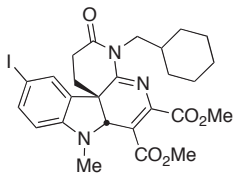
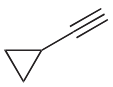
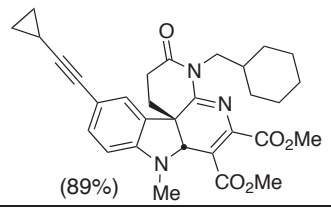
Chart 4. Aryl Iodides and Terminal Alkynes in Cross Coupling Sublibrary (80 Members)



SUMMARY

The intramolecular inverse electron demand Diels-Alder reaction between the electron rich dienophilic double bond of trytamine, tryptophan and related indolic analogues, with tethered electron deficient heteroaromatic azadienes yields indoline alkaloid-type scaffolds which can be used in library synthesis. These natural product-like core structures have been diversified to yield library members with antiviral activities against hepatitis C virus as well as vesicular stomatitis virus. In addition, compounds active against the malaria inducing *P. falciparum* have also been discovered. The readily prepared cycloadduct scaffolds, 8,12,14-triazatetracyclo[7.7.0.^{1,13}.0^{2,7}]hexadeca-2,4,6,10,12-pentaene (**39**), 8,11,12,14-tetraaza-tetracyclo[7.7.0.0^{1,13}.0^{2,7}]hexadeca-2,4,6,10,12-pentaene (**40**), and 5,7,11-triazatetracyclo-[8.7.0.0^{1,6}.0^{12,17}]heptadeca-6,8,12,14,16-pentaen-4-one (**61**) have thereby proven to be promising

Table 2. Representative Cross-Couplings of Aryl Iodides **76** and **76**

Item	Aryl Iodide	Coupling Partner	Product (Yield)[a]
1			 (90%)
2			 (93%)
3			 (92%)
4			 (80%)
5			 (86%)
6			 (89%)

(a) Isolated yields.

structures for library generation and hit discovery in the search for new biological probes and drug candidates.

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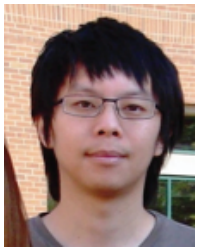

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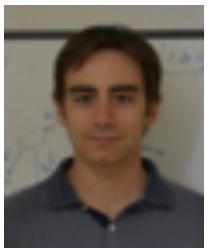

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