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Evolution of Natural Product Scaffolds by Acyl- and Arylnitroso Hetero-Diels−Alder Reactions: New Chemistry on Piperine

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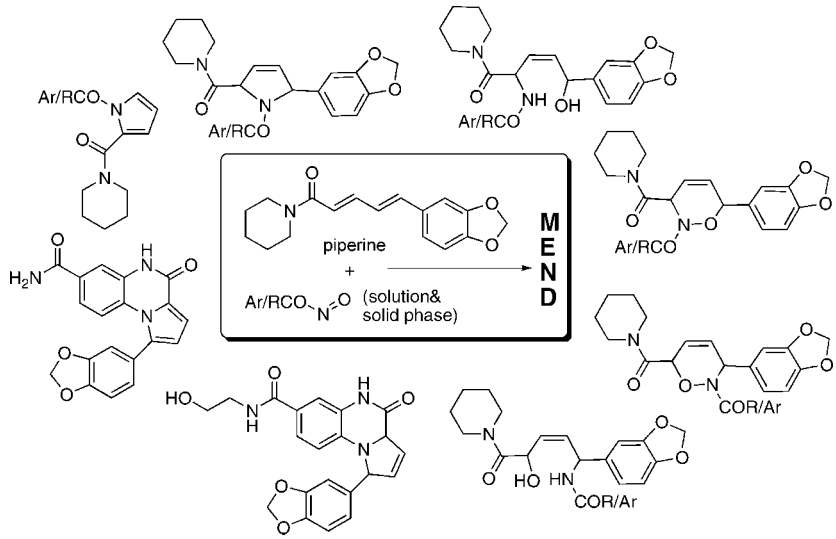

Piperine, a natural product containing a conjugated diene, was reacted with polymer-supported acyl- and arylnitroso dienophiles. The reactions with arylnitroso dienophiles were also carried out in solution. The oxazine rings formed by the corresponding hetero-Diels−Alder reactions were further transformed and novel acyclic as well as heterocyclic derivatives including pyrroles and quinoxalinones were prepared.

Introduction

Transformation of natural products represents one of the main avenues for generating pharmacologically relevant compounds with novel and sometimes altered biological properties. Typical chemical derivatization of natural products such as acylation or alkylation of hydroxy and amino groups results in modification of the periphery of the starting molecules whereas the parent scaffold typically remains mostly unchanged. Although such modifications resulted in many derivatives with desirable biological properties, we sought to develop more substantial transformations of parent molecules under mild and efficient conditions. If able to be accomplished, such transformations would allow natural product scaffolds to be evolved into new platforms for drug discovery.

A large pool of natural product-containing conjugated dienes are available that offer unique opportunities for atypical transformation by hetero-Diels−Alder (HDA) reactions. HDA reactions of diene-containing natural products are attractive from at least three aspects: (i) considerable transformation of the parent molecule topography, (ii) introduction of new heteroatom functionality, as this reaction rigidifies the diene by introducing a six-membered ring with concurrent formation of two carbon−carbon/heteroatom bonds, and (iii) compatibility with a variety of functional groups. The development of the general methodology for transformation of diene-containing natural products to previously unknown scaffolds will enhance opportunities for drug discovery.

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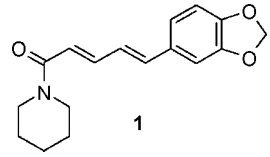

FIGURE 1. Structure of piperine.

We recently described acyl- and arylnitroso polymer-supported HDA reactions using simple dienes as model compounds to address the scope and limitations of these transformations on the solid phase.[1,2] We[3] and others[4,5] also demonstrated that many complex diene-containing natural products readily undergo nitroso cycloaddition reactions (for reviews on nitroso cycloaddition chemistry see refs 6–12). An especially attractive feature of this reaction is that there are no byproducts since both the natural product and the entire nitroso reagent are incorporated into the reaction product, often in high yield under very mild conditions. This provides opportunities for Modular Enhancement of Nature's Diversity (MEND).[3] Here we wish to report our results with piperine, **1**, a natural product containing a conjugated diene motif, that further demonstrates the remarkable versatility of the initial nitroso cycloadduct for use as an evolvable scaffold for MEND. Both solid phase and solution phase elaborations are described.

Piperine (Figure 1) is found as a major component in Asian vine *Piper nigrum* and it is responsible for the spicy flavor of peppers. Piperine has been used in traditional medicine and as an insecticide. Piperine exhibits numerous biological activities, including inhibition of human P-glycoprotein, CYP3A4,[13] and other enzymes important in drug metabolism.[14,15] Piperine stimulates melanocyte proliferation.[16] In addition, piperine enhances the bioavailability of drugs.[17] Bioperine is a marketed product for increasing bioavailability of various dietary supplements.

Results and Discussion

To exploit the diversity of natural products containing conjugated dienes for solid phase syntheses, we chose to immobilize the dienophile rather than developing specific routes for immobilization of individual natural products. Acyl- and arylnitroso HDA adducts were synthesized from polymer-supported nitroso dienophiles and dienes following recently described procedures[1,2] that addressed the preparation of cycloadducts on various linkers and allowed release of target molecules by mild reagents as well as the standard trifluoroacetic acid (TFA) in dichloromethane (DCM) solution, commonly used for cleavage from acid-sensitive linkers.

***N*-H Oxazine.** Synthesis of an HDA adduct without any substitution on the oxazine nitrogen was carried out on a linker that enabled immobilization of a hydroxamate, the precursor of the corresponding transient acylnitroso species, and, after the HDA reaction, release of the cycloadduct by scission of the acyl substituent from the oxazine nitrogen. The acid-stability of HDA adducts of piperine was not known and, while piperine can be considered an electronically mixed diene since it has both an electron-withdrawing acyl group and an electron-rich aromatic group on the diene, based on our previous results,[18] the presence of an electron-rich aromatic ring on the diene terminal carbon indicated potential acid sensitivity. Therefore we carried out the synthesis of the *N*-H oxazine adduct on a silyloxy-based linker cleavable by the mild reagent, TBAF. Briefly, methyl 4-hydroxybenzoate was reacted with an equimolar amount of diisopropyldichlorosilane and the resulting monosilyl ether was added to hydroxymethyl polystyrene−1% divinylbenzene support to provide resin **2** (Scheme 1). The polymer-supported ester, **2**, was reduced by DIBAL and the 4-silyloxybenzyl alcohol **3** was reacted with CDI and subsequently with hydroxylamine to afford the required intermediate *N*-hydroxy carbamate, **4**, for subsequent HDA reactions. Oxidation to the corresponding nitroso species was carried out by treatment with n-Bu_4NIO_4 in the presence of piperine. The cycloaddition products, **6**, were cleaved from the derivatized solid support **5** by TBAF and analyzed.

Analysis of the crude product after cleavage by TBAF revealed the presence of two major components exhibiting identical mass spectra that corresponded to two closely eluting regioisomers **6a** and **6b** in a 7:3 ratio. In all piperine HDA adducts described in this paper isomer "**a**" always refers to the structure that contains the carboxamide residue connected to the C3 carbon and the phenyl ring attached to the C6 oxazine carbon. In our previous study of HDA reactions on the solid phase with asymmetric dienes we observed predominant formation of one regioisomer. The major isomer contained the bulkier group on the C6 carbon, irrespective of the electronic properties of the group.[2] Piperine contains two sizable groups on both diene termini, the electron-rich phenyl group and electron-withdrawing carboxamide. The fact that both isomers were formed documented the prevalence of steric effects over electronic effects.

Because the silyloxy linker is acid sensitive, we also released the isomeric HDA adducts using standard TFA conditions to address the stability of the new piperine-derived oxazine ring. In 50% TFA in DCM, the major isomer, **6a**, partially decomposed. The alkoxyphenyl substituent stabilized carbocation **8** formed by acid-mediated cleavage of the C−O bond caused decomposition of the oxazine ring. When we exposed the mixture of two resin-bound isomers **5** to TFA containing the carbocation scavenger triethylsilane (TES), isomer **6b** was found to be stable, whereas the oxazine ring of isomer **6a** was cleaved and intermediate carbocation **8** was quenched by TES to form

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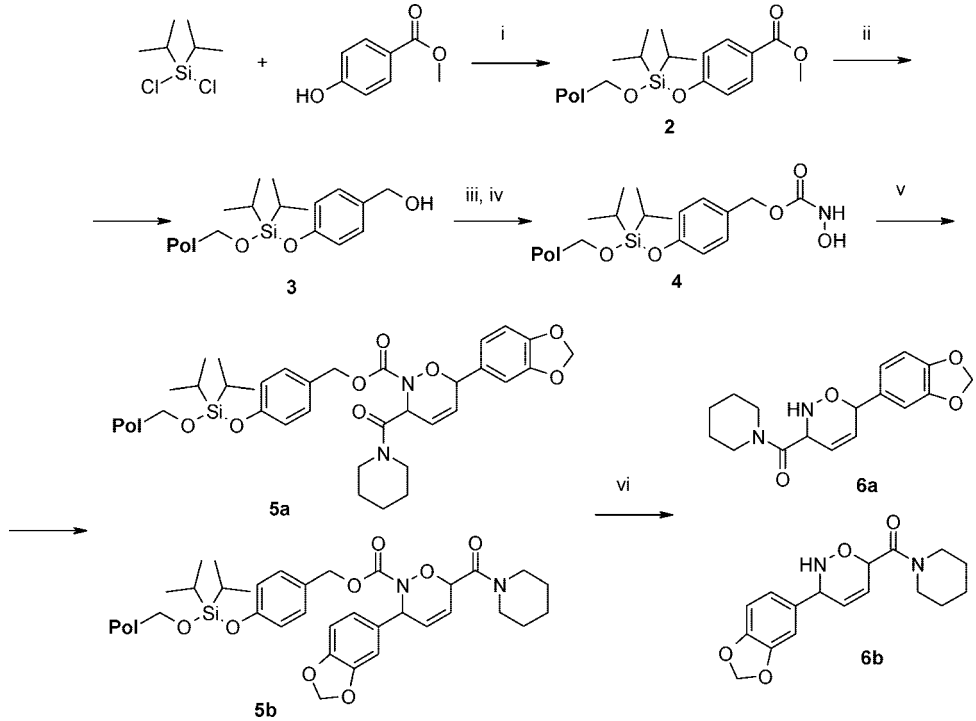
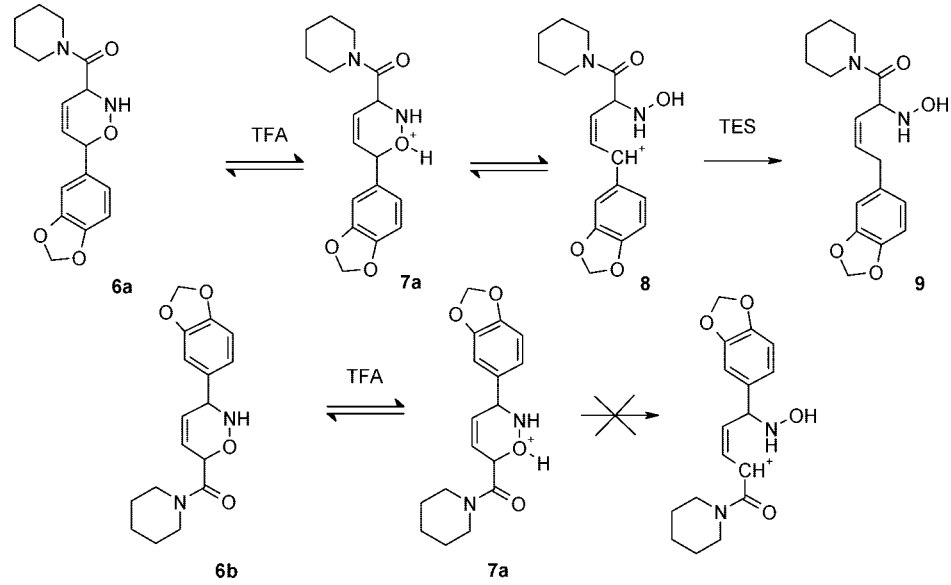
SCHEME 1. Synthesis of *N*-H Oxazine from Piperine on a Solid Support with a Silyloxy-Based Linker[a]



[a] Reagents and conditions: (i) TEA, DMAP, DCM, rt, 1 h, then hydroxymethyl resin, rt, 1 h; (ii) DIBAL, THF, 1 h; (iii) CDI, pyridine, DCM, rt, 1 h; (iv) $NH_2OH \cdot HCl$, pyridine, rt, 2 h; (v) piperine, 0.1 M n-Bu_4NIO_4, DCM, rt, 1 h; (vi) 0.1 M TBAF, THF, rt, 30 min, 13% (yields for all solid-phase syntheses are based on initial loading of a linker).

SCHEME 2. Preparation of a Piperine-Derived Acyclic Hydroxylamine[a]



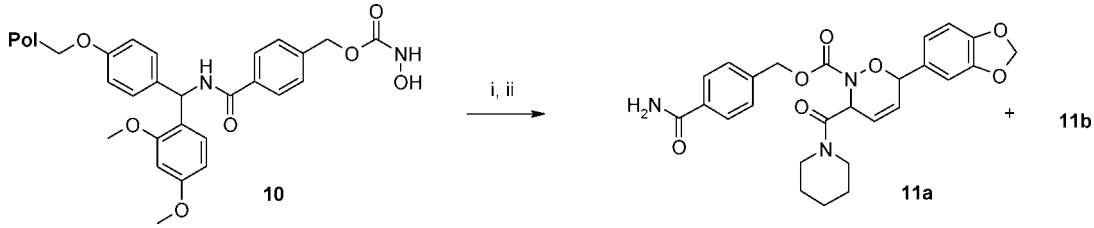
[a] Reagents and conditions: (i) 45% TFA, 10% TES, DCM, 30 min, 19%.

acyclic product **9** (Scheme 2). The different fates of the regioisomers upon treatment with acidic conditions are consistent with the structural assignment of the isomers as acid treatment of **6a** would be expected to more readily generate the highly stabilized benzylic cation intermediate.

***N*-Acyloxazine.** Acylnitroso HDA adducts with *N*-acylated oxazine were prepared in an analogous manner. Thus, 4-hydroxymethylbenzoic acid was immobilized through a Rink amide linker and the resin-bound benzyl alcohol was converted to hydroxamate **10** (Scheme 3). The Rink resin facilitated acid-mediated cleavage of the product as a carboxamide **11**, while leaving the acyl substituent at the oxazine nitrogen intact due to the electron-withdrawing effect of the carboxamide group. LCMS analysis of the crude product showed only one peak; however, the ^1H and ^{13}C NMR spectra of HPLC purified material revealed the presence of two regioisomers of **11** in an 8:2 ratio.

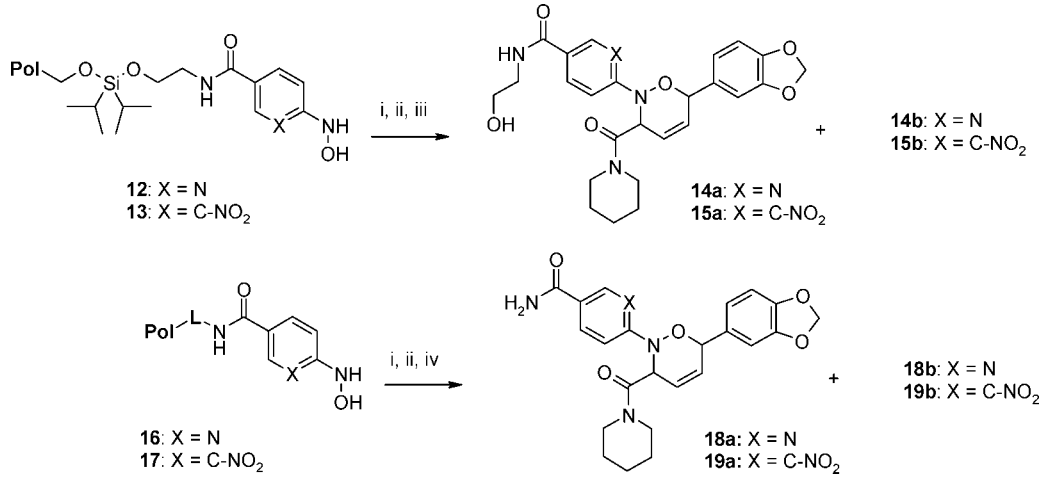
Arylnitroso HDA Adducts. The arylnitroso dienophiles were separately prepared from 4-fluoro-3-nitrobenzoic and 6-fluoronicotinic acid immobilized by using two different linkers: (i) via ethanolamine to a silyloxy linker that would be cleavable by TBAF and (ii) by acylation of the Rink resin that would be

SCHEME 3. Synthesis of *N*-Acyl Oxazine from Piperine on Rink Resin[a]



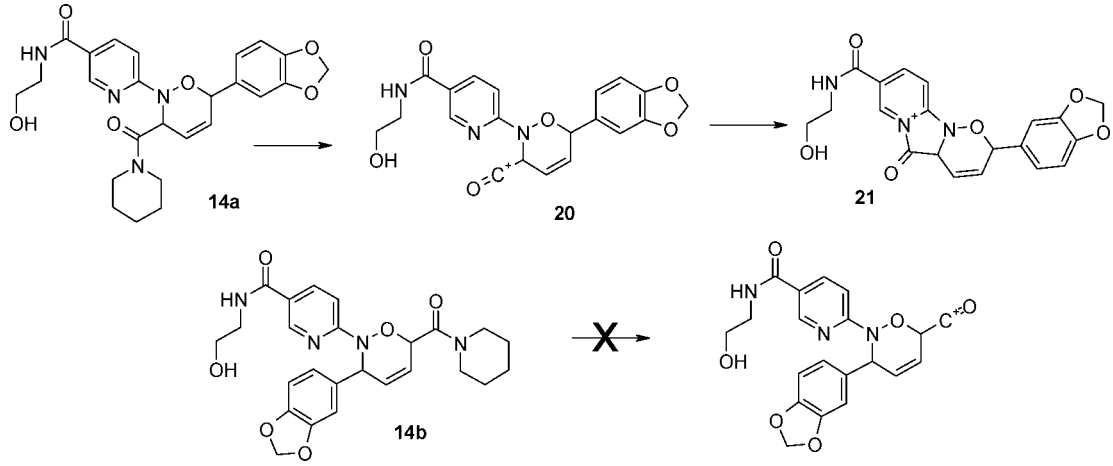
[a] Reagents and conditions: (i) piperine, 0.1 M n-Bu_4NIO_4, DCM, rt, 1 h; (ii) 50% TFA, DCM, 30 min, 23%.

SCHEME 4. Synthesis of (Hetero)arylnitroso HDA Adducts[a]



[a] Reagents and conditions: (i) 0.1 M n-Bu_4NIO_4, DCM, rt, 1 h; (ii) piperine, DCM, overnight; (iii) 0.1 M TBAF, THF, rt, 30 min, 33% (**14a**), 13% (**14b**); (iv) 50% TFA, DCM, 30 min, 33% (**18a**), 13% (**18b**).

SCHEME 5. Fragmentation of *N*-Pyridyl Oxazine from Piperine

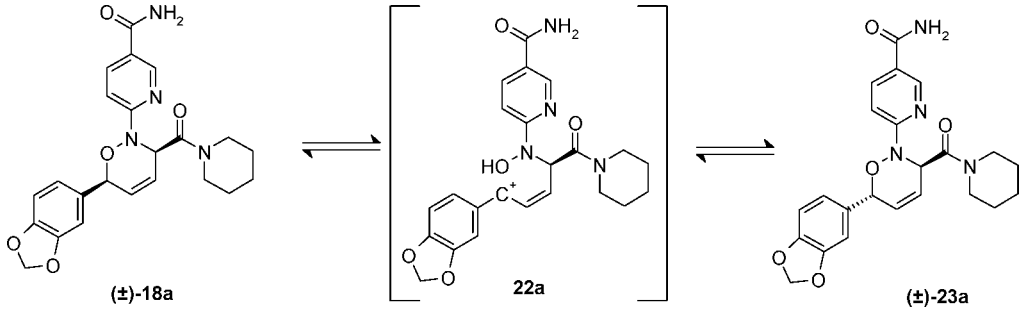


cleavable by TFA (Scheme 4). Fluorine was replaced by nucleophilic aromatic substitution with hydroxylamine followed by oxidation to the corresponding nitroso species with tetrabutylammonium periodate prior to HDA reaction with piperine.[2] Both dienophiles represent electron-deficient arylnitroso species. The major differences were the presence of a nitro group ortho to the nitroso group in HDA adducts **15** and **19** and the presence of protonatable pyridyl nitrogen in cycloadducts **14** and **18**, structural differences that turned out to have a more profound effect than initially expected.

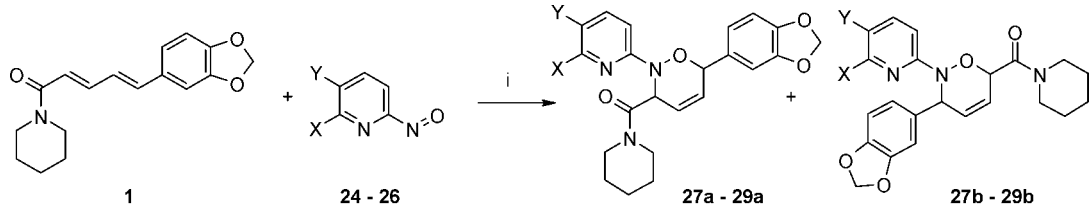
***N*-Pyridyloxazine. Solid-Phase Experiments.** Reaction of the pyridylnitroso dienophiles with piperine afforded, after cleavage with TBAF, two regioisomers in a 7:3 ratio. The mass spectrum of one isomer revealed the presence of a very intense fragment at m/z 396.2, in addition to a molecular ion at $[M + H]^+$ 481.2. This fragment corresponded to acylium ion, **20**, formed by scission of the amide bond (Scheme 5). Because isomer **14a** can stabilize the acylium ion by formation of a five-membered acylpyridinium ring, **21**, we concluded that the compound that afforded the fragment corresponded to isomer **14a** whereas the other isomer **14b** did not have any stabilizing contribution of the neighboring groups.

Cleavage with 10% TFA in DCM afforded a mixture of isomers in a 74:26 ratio that favored isomer **14a**. Increasing the concentration of TFA to 50% changed the ratio to 64:36 indicating decomposition of isomer **14a**. Addition of TES to

SCHEME 6. Epimerization of One Enantiomeric HDA Adduct in TFA



SCHEME 7. Preparation of Piperine HDA Adducts in Solution[a]



24, 27: X=Me, Y=H
25, 28: X=H, Y=Cl
26, 29: X=H, Y=Br

[a] Conditions: (i) 60 °C, 3 h, then rt, 16 h, 86% (**27**), 73% (**28**), 64% (**29**).

the TFA-containing cleavage cocktail did not change the isomer ratio, although formation of the reduced compound was not observed.

HDA adducts **18**, without the ethanolamine appendage, prepared from the 6-fluoronicotinic acids coupled to Rink resin, were found to behave analogously. Cleavage with 10% TFA provided the expected two regioisomers. Increasing the concentration to 50% TFA caused formation of a third component, which exhibited a mass spectrum identical with that of isomer **18a**. The ^{1}H NMR spectrum contained the same pattern of proton resonances, but slightly shifted in the 5−7 ppm region, indicating formation of diastereomers **23a**. Thus, apparently the C−O bond was cleaved in the TFA solution and the initially formed carbocation **22a** recombined to give an oxazine; however, at this stage the stereochemistry of the C6 oxazine carbon was lost and a diastereoisomer mixture at the benzylic carbinol carbon of **23a** was formed (Scheme 6).

Solution-Phase Experiments. In parallel with solid-phase syntheses we carried out the HDA reaction with both components in solution. Reaction of piperine with 2-methyl-6-nitrosopyridine, **24**, 5-bromo-2-nitrosopyridine, **25**, and 5-chloro-2-nitrosopyridine, **26**, prepared according to Taylor et al.,[19] afforded the HDA cycloadducts as a 2:1 mixture of regioisomers (Scheme 7). The mixture of two regioisomers of **27** was analyzed by chiral HPLC and four compounds corresponding to two pairs of enantiomers (ratio 1:1) from both regioisomers were detected. Purification of regioisomers by flash column chromatography was unsuccessful, resulting in isolation of a mixture. Both regioisomers were isolated by semipreparative HPLC and characterized by NMR and MS spectra. The mass spectra of two regioisomers **27a** and **27b** revealed a fragmentation pattern comparable to MS spectra of HDA adducts **14a** and **14b**. Isomer **27a** afforded, in addition to the molecular ion, a strong signal at *m*/*z* 323.4, corresponding to a fragment formed by scission of the amide bond, analogously to isomer **14a**. Unequivocal assignment of the structure of individual regioisomers was made possible by X-ray analysis. The major regioisomer crystallized and its X-ray analysis confirmed the structure as that shown for **27a**.

TABLE 1. Relative Ratio of Regioisomers As a Function of Catalyst Quantity[a]

entry	Cu catalyst, equiv	ratio (**27a**:**27b**)
1	none	2:1
2	0.20	4:1
3	0.50	8:1
4	0.75	12:1
5	1.0	16:1

[a] Reagents and conditions: $(MeCN)_4Cu(I)PF_6$, 2-methyl-6-nitrosopyridine (1 equiv), rt, 30 min, then piperine (1 equiv), rt, 16 h.

To determine whether the regioselectivity could be enhanced, we carried out the HDA reaction in the presence of a copper catalyst, tetrakis(acetonitrile)copper(I) hexafluorophosphate.[20] The crude products were analyzed by normal phase chiral HPLC. A control sample with no copper catalyst was also included and showed a 2:1 ratio of the major isomer, **27a**, to the minor isomer, **27b**. Chiral analytical HPLC analysis revealed a 1:1 ratio of each set of enantiomers for the given regioisomer, as expected (the individual compounds were not isolated). An increased amount of the copper catalyst enhanced the regioselectivity in favor of the major isomer, while the ratio of the enantiomers expectedly remained unchanged for each pair. The results are summarized in Table 1.

Although the nitroso HDA adducts are interesting compounds per se, and exhibit a range of biological activities,[1] they provide an attractive scaffold for further transformations. Kouklovsky

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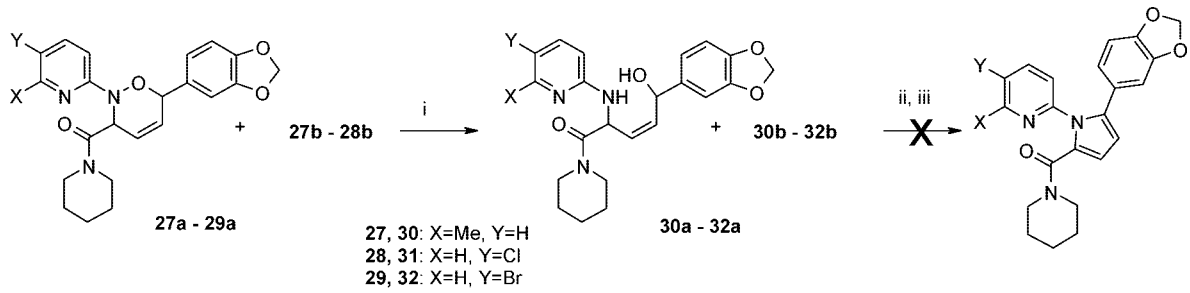
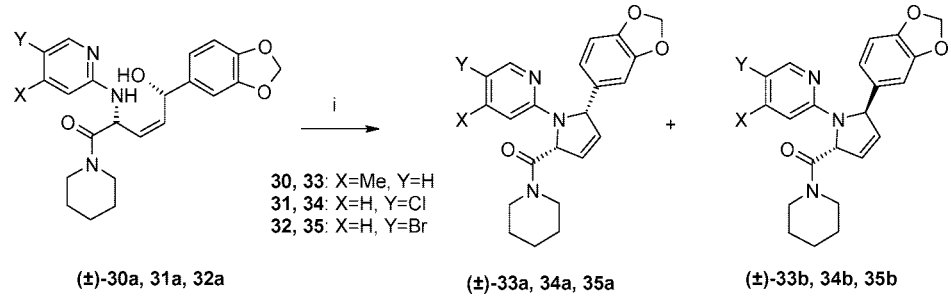
SCHEME 8. Transformation of HDA Adducts (Scaffold Evolution)[a]



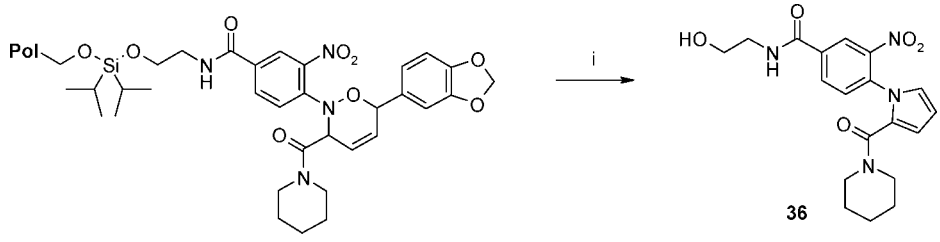
[a] Reagents and conditions: (i) $Mo(CO)_6$, reflux 6 h in MeCN:H_2O (16:1), 47% (**30a**), 40% (**31a**), 38% (**30**); (ii) MnO_2, DCM, 30 °C, 16 h; (iii) TFA, DCM, 45 min.

SCHEME 9. Synthesis of Dihydropyrroles (Only One Stereoisomer Depicted)[a]



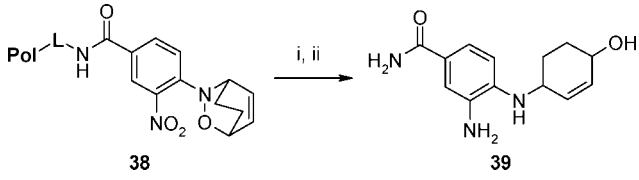
[a] Conditions: (i) 10% TFA/DCM, 45 min, 41% (**33a**), 53% (**33b**), 36% (**34a**), 24% (**34b**), 42% (**35a**), 15% (**35b**).

SCHEME 10. Ring Contraction of *N*-Nitroaryloxazine to a Pyrrole[a]



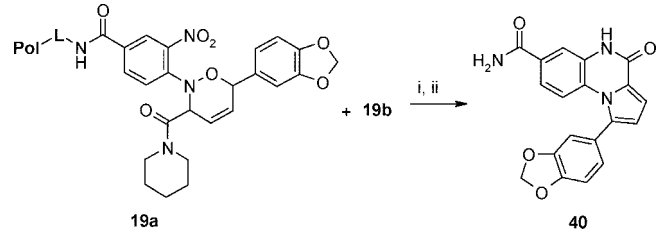
[a] Reagents and conditions: (i) 50% TFA, DCM, 30 min, 19%.

SCHEME 11. Treatment of Model HDA Adduct with Tin(II) Chloride[a]



[a] L refers to Rink linker. Reagents and conditions: (i) $SnCl_2 \cdot 2H_2O$, NMP, rt, 2 h; (ii) 50% TFA, DCM, 30 min, 23%.

SCHEME 12. Tin(II) Chloride Reduction of Piperine HDA Adduct on Rink Linker[a]



[a] L stands for Rink amide linker. Reagents and conditions: (i) $SnCl_2 \cdot 2H_2O$, DIEA, NMP, rt, 2 h; (ii) 50% TFA, DCM, 30 min, 21%.

et al.[21] have shown that reduction of the N−O bond in HDA cycloadducts provides an allylic alcohol that can be subsequently oxidized and cyclized to pyrroles. Accordingly, we reduced the N−O bond in the HDA adducts **21** by $Mo(CO)_6$[22] to afford amino alcohols **30** (Scheme 8). The major regioisomer **30a** was isolated and fully characterized. HDA adducts with nitroso compounds **25** and **26** were also subjected to reaction with $Mo(CO)_6$ and readily provided the N−O reduced compounds **31** and **32**. This chemistry demonstrates that further derivatives can be made starting from different nitroso species.

To convert the amino alcohols **30** to pyrroles, we subjected the major isomer of the N−O bond reduced compound **30a** to MnO_2 in refluxing dichlormethane to oxidize the alcohol; however, the formation of the cyclized product was not observed. The original paper[21] described pyrrole synthesis from *N*-Boc oxazines whereas our substrates contained a heteroaryl substituent. The failure to form the pyrrole derivative with our substrates suggested structural dependence on the *N*-substitution of the amino alcohol toward the transformation.

An alternative route to synthesize pyrrole derivatives was initiated by exposure of the N−O bond-reduced cycloadduct **30a** to a 50% TFA/DCM. Analysis by LCMS confirmed the

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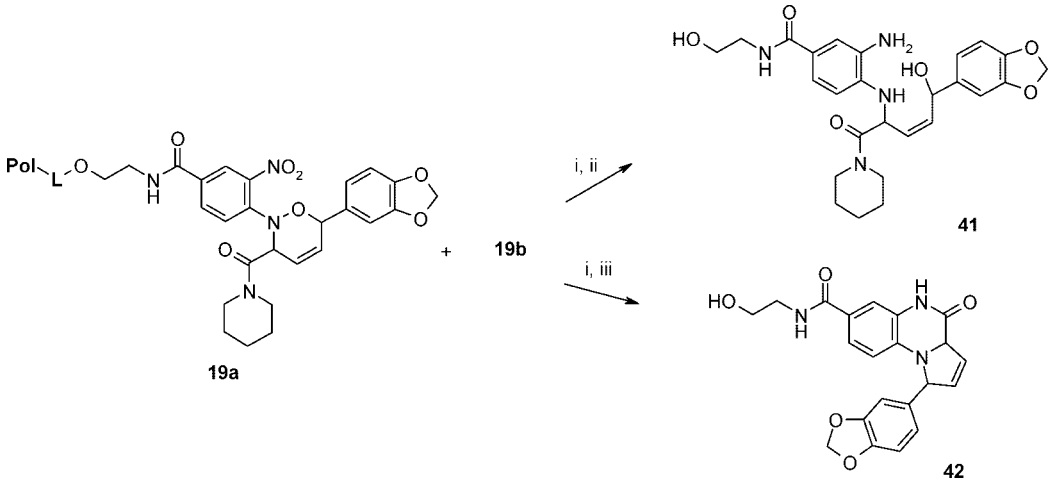
SCHEME 13. Tin(II) Chloride Reduction of Piperine HDA Adduct on Silicon Linker[a]



[a] L stands for silicon linker (for structure, see **13**). Reagents and conditions: (i) $SnCl_2 \cdot 2H_2O$, NMP, rt, 16 h; (ii) 0.01 M TBAF, THF, 30 min; 31%; (iii) 50% TFA, DCM, 30 min, 42%.

presence of two components with a mass spectrum corresponding to the 2,5-dihydro compounds **33a** and **33b** (Scheme 9). ^{1}H NMR lent further support for formation of **33a** and **33b**. Ultimately, X-ray data were obtained for compound **33a** as the TFA salt and confirmed its structure. Encouraged by this result, amino alcohols **31a** and **32a** were subjected to a 50% TFA/DCM solution and 2,5-dihydropyrroles **34** and **35** were obtained, isolated, and characterized.

***N*-Nitroaryloxazine.** Two regioisomers of the *N*-nitroaryloxazine cycloadduct **15** were obtained after TBAF cleavage from the silicon linker. Treatment of the nitroarylnitroso HDA adducts with 50% TFA was accompanied by ring contraction and formation of pyrrole derivative **36** (Scheme 10). The aryl substituent was not present on the pyrrole ring, but replaced by a proton, presumably by electrophilic aromatic substitution. Transformation of oxazine derivatizes to pyrroles in acidic media has already been reported.[23] The presence of TES in 50% TFA did not afford the reduced compound, but the same pyrrole derivative was detected in the cleaved sample. The same ring contraction and formation of pyrrole **37** was observed when the HDA adduct was synthesized on Rink resin and cleaved by 50% TFA in DCM.

Tin(II) Reduction. Tin(II) chloride dihydrate is a frequently used reagent for reduction of polymer-supported nitroaromatic compounds to anilines.[24] In a model experiment, we treated HDA adduct **38** with a solution of tin(II) chloride dihydrate in NMP and observed formation of one major product, **39**, indicating that both the nitro group and the N−O bond of the oxazine had been reduced to give an amino cyclohexenol derivative (Scheme 11). This constitutes a net 1,4-aminohydroxylation reaction.

Encouraged by these results, we subjected HDA adducts **19** to reducing conditions. Treatment with 1 M $SnCl_2 \cdot 2H_2O$, 1 M DIEA in NMP for 2 h afforded, after cleavage by 50% TFA, 5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one derivative **40** (Scheme 12) as the major component.

The cyclization to quinoxalinone derivative **40** was not spontaneous and it was induced by TFA, required for cleavage of the target compound from the Rink linker. To isolate the linear precursor, the arylnitroso species was linked to the resin via a silicon-based linker that facilitated mild cleavage of products by TBAF. After the Tin(II)-mediated reduction and TBAF cleavage, we isolated compound **41**, resulting from reduction of both the nitro group as well as the N−O oxazine bond (Scheme 13). Cleavage with TFA yielded 1,3a-dihydro-5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one derivative **42**, unlike the HDA adducts **19** on Rink linker that afforded pyrrole derivatives. The cause of N−O bond reduction in the case of the HDA adduct on silicon linker remains to be explained.

Reduction of the N−O bond by tin(II) chloride was unsuccessful on acylnitroso- and pyridylnitroso-derived HDA adducts. After overnight exposure to a 2 M solution in NMP, formation of amino alcohols was not observed. When compared to the model experiment with cyclohexadiene adduct, **38**, the piperine cycloadducts contained only one six-membered ring, whereas the cyclohexadiene adduct **38** is a bicyclic ring system. Breaking the N−O bond in the two-ring system still preserved the carbocyclic six-membered ring, whereas the same reaction on the piperine adduct would result in formation of an acyclic compound. Cleavage of the N−O bond in single ring oxazines requires more forcing conditions and was typically carried out by zinc in acid or use of $Mo(CO)_6$ as described earlier.

Compounds prepared on the solid phase were subjected to a panel of biological assays that included tests for antimicrobial, antiinflammatory, antiproliferative, and cytotoxic activities. Assay details are described in the Supporting Information. Antimicrobial activity was assayed by agar diffusion tests, using a range of Gram-positive and Gram-negative bacteria, yeasts, and fungi. Compound **40** exhibited weak activity against Gram-positive bacteria and inhibited growth of mycobacteria. Compound **40** also exhibited moderate antiproliferative efficacy against K-562 cells and moderate antiproliferative efficacy on Huvec cells. Antiinflamatory activity in the horseradish peroxidase assay, comparable to the standard, *N*-acetylcysteine, was exhibited by compounds **39** and **41**.

Conclusion

Four different acyl- and arylnitroso HDA cycloadducts with piperine were prepared by using polymer-supported dienophiles. Related solution-phase chemistry was also performed to dem-

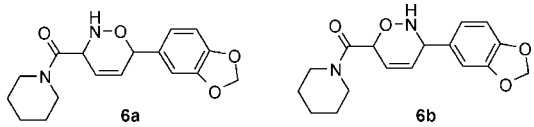
(23) Firl, J. *Chem. Ber.* **1968**, *101*, 218–225.
(24) Morales, G. A.; Corbett, J. W.; DeGrado, W. F. *J. Org. Chem.* **1998**, *63*, 1172–1177.

onstrate the utility of the methodology and to confirm product structures. The type of *N*-substituent had a remarkable affect on the results of attempted further modifications of the HDA adducts. The HDA adducts were further transformed to yield novel acyclic products as well as heterocycles including pyrrole and quinoxalinone derivatives.

Experimental Section

Syntheses of resin-bound acyl- and arylnitroso dienophiles and their HDA reactions were described in detail in our previous papers.[1,2] Here we report analytical data of representative piperine HDA adducts.

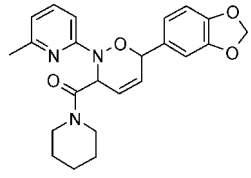
(6-Benzo[1,3]dioxol-5-yl-3,6-dihydro-2*H*-[1,2]oxazin-3-yl)piperidin-1-yl-methanone and Its Regioisomer (6). Yield (HPLC purified)



33 μmol (13%) of two regioisomers 7:3. ESI-MS *m/z* 317.2, $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.72−6.96 (m, 3 H), 6.02−6.11 (m, 1 H), 6.02 (s, 2 H), 5.96−6.00 (m, 1 H), 5.06−5.20 (m, 1 H), 4.49−4.66 (m, 1 H), 3.39−3.54 (m, 4 H), 1.37−1.67 (m, 6 H). Isomer **6a**: ^{13}C NMR (75 MHz, DMSO-d_6) δ major isomer 167.3, 147.3, 147.3, 132.9, 128.4, 124.3, 122.2, 108.8, 107.9, 101.1, 75.1, 54.6, 45.8, 42.4, 26.1, 25.3, 24.0. Isomer **6b**: ^{13}C NMR (75 MHz, DMSO-d_6) δ minor isomer 168.2, 147.2, 146.8, 132.8, 128.9, 124.2, 121.6, 108.7, 108.2, 101.0, 70.1, 57.5, 45.8, 42.3, 26.3, 25.3, 24.0. HRMS (FAB) *m/z* calcd for $C_{17}H_{21}N_2O_4$ 317.1501, found 317.1494.

Piperine Cycloadduct 27. Piperine (116 mg, 0.407 mmole) and 2-methylnitrosopyridine (74 mg, 0.610 mmol) were dissolved in 1 mL of MeOH and the resulting mixture was heated to 60 °C for 3 h, then stirred for 16 h at room temperature. The solvent was removed under reduced pressure. Flash chromatography (silica gel; eluted with 50% EtOAc/hex) provided 129.5 mg (86%) of **27a** and **27b** in a 2:1 ratio as a yellow oil, and 63 mg of the product was purified by prep HPLC (70% MeCN/H_2O) to yield 45.5 mg of two isomers **27a** and **27b** (29.1 and 16.4 mg, respectively). ESI-MS *m/z* 408, $[M + H]^+$ for both isomers.

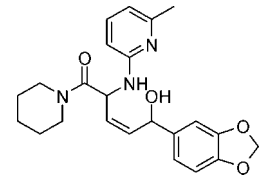
(6-(Benzo[*d*][1,3]dioxol-5-yl)-2-(6-methylpyridin-2-yl)-3,6-dihydro-2*H*-1,2-oxazine-3-yl)piperdin-1-yl)methanone (27a). ^{1}H NMR



(300 MHz, $CDCl_3$) δ 7.51 (dd, J = 7.5 Hz, 8.1 Hz, 1 H), 7.08 (d, J = 1.5 Hz, 1 H), 7.00 (d, J = 8.1 Hz, 1 H), 6.99 (dd, J = 7.8, 1.5 Hz, 1 H), 6.81 (d, J = 7.8 Hz, 1 H), 6.65 (d, J = 7.5 Hz, 1 H), 6.17 (ddd, J = 7.2, 5.1, 2.4 Hz, 1 H), 6.07−6.04 (m, 1 H), 5.96−5.95 (m, 1 H), 5.93−5.92 (m, 1 H), 5.38−5.36 (m, 1 H), 3.68−3.49 (m, 4 H), 2.41 (s, 1 H), 1.61−1.58 (m, 6 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 158.0, 156.7, 148.3, 148.0, 138.4, 131.8, 128.9, 123.4, 122.8, 115.4, 109.5, 108.4, 106.5, 101.3, 53.9, 46.9, 43.8, 26.6, 25.9, 24.9, 24.6. HRMS $C_{23}H_{25}N_3O_4$ calcd 408.1923, found 408.1920.

N−O Bond Reduction of Piperine Cycloadducts 27. A solution of cycloadducts **27** (100 mg, 0.245 mmol) in 14:1 MeCN:H_2O (1.5 mL) was charged with $Mo(CO)_6$ (64.8 mg, 0.245 mmol). The flask was equipped with a cold-water condenser and heated to reflux. The cooled mixture was filtered and concentrated to a dark oil. Flash chromatography (silica gel; eluted with 30% EtOAc/hex) provided 47 mg (47%) of a yellow solid.

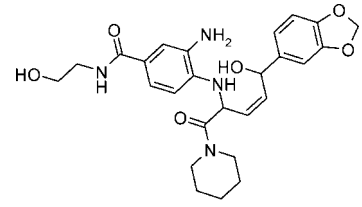
(*Z*)-5-(Benzo[*d*][1,3]dioxol-5-yl)-5-hydroxy-2-(6-methylpyridin-2-ylamino)-1-(piperidin-1-yl)pent-3-en-1-one (30a). ^{1}H NMR (300



MHz, $CDCl_3$) δ 8.04 (br s, 1 H), 7.26 (dd, J = 8.4, 7.2 Hz, 1 H), 6.81−6.70 (m, 2 H), 6.43 (dd, J = 10.5, 6.9 Hz, 1 H), 6.34 (dd, J = 15 Hz, 4.5 Hz, 2 H), 6.00 (d, J = 6.3 Hz, 1 H), 5.93 (s, 2 H), 5.76 (dd, J = 11.7, 3.6 Hz, 1 H), 5.42 (dd, 4, 4 Hz, 1 H), 5.32 (ddd, J = 22, 11.7, 2.4 Hz, 1 H), 3.78−3.74 (m, 2 H), 3.61−3.44 (m, 2 H), 2.06 (s, 3 H), 1.71−1.57 (m, 6 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.2, 156.3, 147.8, 147.0, 138.4, 138.2, 133.8, 127.4, 120.7, 112.2, 108.2, 108.1, 107.9, 101.1, 76.8, 74.1, 49.0, 46.3, 43.9, 26.2, 25.9, 24.7. HRMS (FAB) *m/z* $C_{23}H_{27}N_3O_4$ calcd 410.2080, found 410.2096.

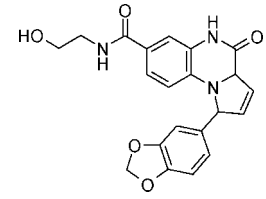
Reduction of the Nitro Group. Resins **19** and **25** (200 mg) were washed 3× with DMF and 2 mL of a solution of 1 M tin chloride dihydrate and 1 M DIEA, prepared under nitrogen bubbling, was added to the resin and the resin slurry was shaken for 2 h. The resin was washed very thoroughly 7× with DMF and 3× with DCM.

3-Amino-4-[4-benzo[1,3]dioxol-5-yl-4-hydroxy-1-(piperidine-1-carbonyl)but-2-enylamino]-*N*-(2-hydroxyethyl)benzamide (41). Yield (HPLC purified) 9.6 umol (31%). ESI-MS *m/z* 497.5 $[M +$



$H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.88 (t, J = 5.52 Hz, 1 H), 7.10 (d, J = 1.93 Hz, 1 H), 7.05 (dd, J = 8.42, 2.07 Hz, 1 H), 6.91 (s, 1 H), 6.85 (d, J = 0.83 Hz, 2 H), 6.51 (d, J = 8.01 Hz, 1 H), 5.98 (s, 2 H), 5.66−5.81 (m, 1 H), 5.38−5.53 (m, 3 H), 5.24−5.36 (m, 2 H), 4.62−4.77 (m, 3 H), 3.41−3.50 (m, 2 H), 3.32−3.41 (m, 4 H), 3.26 (q, J = 5.80 Hz, 2 H), 1.49 (br s, 2 H), 1.30 (dd, J = 14.78, 7.32 Hz, 4 H). HRMS (FAB) *m/z* calcd for $C_{26}H_{32}N_4O_6$ $[M]^+$ 496.2322, found 496.2330.

1-Benzo[1,3]dioxol-5-yl-4-oxo-1,3a,4,5-tetrahydropyrrolo[1,2-*a*]quinoxaline-7-carboxylic acid (2-hydroxyethyl)amide (42). Yield



(HPLC purified) 13.2 umol (42%). ESI-MS *m/z* 394.3 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.56 (s, 1 H), 8.06 (t, J = 5.80 Hz, 1 H), 7.32 (d, J = 1.66 Hz, 1 H), 7.24 (dd, J = 8.42, 1.80 Hz, 1 H), 6.82 (d, J = 7.73 Hz, 1 H), 6.74 (dd, J = 8.01, 1.66 Hz, 1 H), 6.60 (d, J = 1.38 Hz, 1 H), 6.52 (d, J = 8.56 Hz, 1 H), 6.19−6.27 (m, 1 H), 5.99−6.08 (m, 1 H), 5.94 (d, J = 6.91 Hz, 2 H), 5.65−5.74 (m, 1 H), 4.76−4.85 (m, 1 H), 4.66 (t, J = 5.52 Hz, 1 H), 3.38−3.48 (m, 2 H), 3.13−3.28 (m, 2 H). ^{13}C NMR

spectrum (75.4 MHz, DMSO-d_6) δ 166.1, 165.1, 147.5, 146.7, 134.4, 133.9, 133.3, 127.9, 124.7, 124.0, 121.5, 121.0, 114.9, 113.6, 108.1, 107.1, 101.1, 68.4, 65.5, 59.9, 42.0. HRMS (FAB) *m*/*z* calcd for $C_{21}H_{20}N_3O_5$ 392.1246, found 392.1265.

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Supporting Information Available: Details of experimental procedures, spectroscopic data for synthesized compounds, and copies of NMR spectra. Crystallographic results are deposited in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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