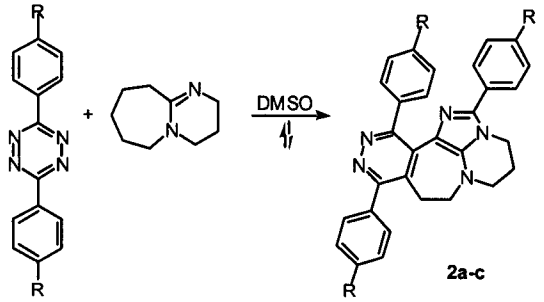
(1) **Synthesis of Fused Tetraheterocyclic Azepines**: The reaction of amidines with 1,2,4,5-tetrazines has previously been reported to give 1,2,4-triazines **III**. In this reaction, benzamidine was employed, which allows for elimination of ammonia after initial cycloaddition. In this study, we were curious if an N-substituted amidine, such as DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), would deliver **IV** which could not achieve aromatization by elimination. In our search for unaromatized dihydrotriazine **IV**, attempts conducted at lower temperatures (rt to refluxing MeOH) showed no reaction between 3,6-diphenyl-1,2,4,5-tetrazine **1a** (**Scheme 1**) and DBU. When the reaction was carried out in triglyme at reflux (216 °C) the reaction did proceed and the intense purple hue of the tetrazine gradually disappeared. Isolation of the reaction product, however, did not yield **IV** but rather **2a**, which contains an unexpected 5,6,6,7-fused heterocyclic ring system.

We have examined this interesting finding in an attempt to determine its scope and limitations. Mechanisic considerations and experimental observations (i.e., minimized side-products as judged by TLC indicated that a 5:1 stoichiometric ratio of tetrazine to DBU was most effective. Next, the removal of the triglyme from the reaction mixture was problematic and a more efficient method of purification was sought. When the reaction was conducted in refluxing *m*-xylene (bp 138-9 °C), it was incomplete (presence of tetrazine by TLC) even after two days. The best solvent found for the reaction and subsequent purification of product **2a** was DMSO (bp 189 °C). The reaction of other s-tetrazines [di-(4-methylphenyl)tetrazine **1b** and di-(4-methoxyphenyl)tetrazine **1c**] with DBU were also examined and found to yield the corresponding azepine heterocycles **2b** and **2c**. Isolated yields of **2 a-c** ranged from 38-45%. The reactivity of 3,6-di-(2-pyridyl)-1,2,4,5-tetrazine was much greater; employing toluene at reflux resulted in the starting tetrazine being consumed within 1 h. Unfortunately, any material that was formed decomposed quickly and could not be isolated.



Scheme 1: Imidazopyridazinopyrimidinoazepines from reaction of tetrazines with DBU.

Products **2 a-c**, with their 4-aminoimidazole moiety, appeared red to orange on silica gel and their structures were established by spectroscopic data. In addition, the structure of **2a** was verified by x-ray crystallography (**Figure 1**).

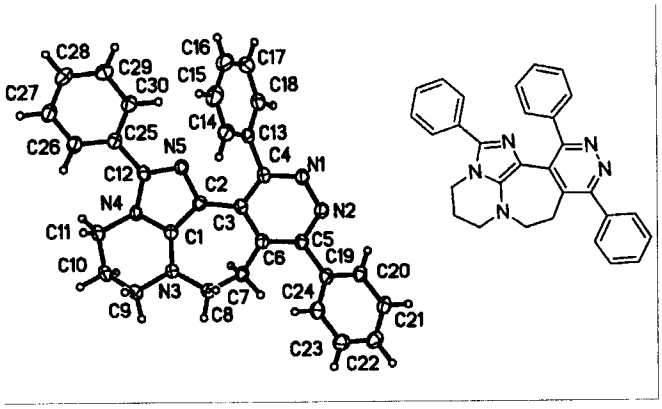


Figure 1: X-Ray crystal structure of **2a.**

Formation of **2** can be rationalized by a mechanism which involves intermediates shown in **Figure 2**. In this process, DBU acts as both reactant and reducing agent while the tetrazine acts as both reactant and oxidizing agent. Nazer and Haddadin observed the reductive effect of DBU on *o*- and *p*-nitrobenzaldehydes, which were reduced to the corresponding aminobenzoic acids. Furthermore, Sauer *et al.* recently described the use of 1,2,4,5-tetrazine as both reactant and oxidizing agent.

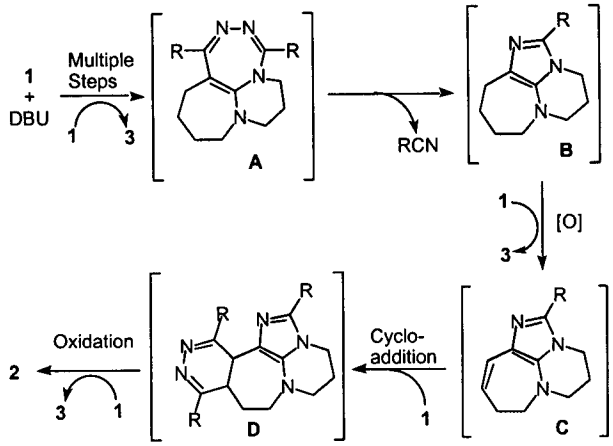
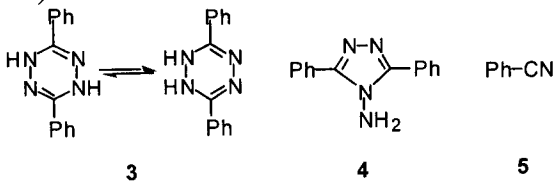


Figure 2: Mechanistic rationale for **1** + DBU → **2.**

While it is possible that triazepine ring contraction with extrusion of benzonitrile could succeed tetrahydroazepine oxidation and cycloaddition, this is not likely given the relative instability of triazepines at these elevated reaction temperatures (189 °C). Intermediate **D** undergoes subsequent oxidation to **2** with another molecule of tetrazine acting as the oxidizing agent. Attempts to isolate any of these or other intermediates were unsuccessful. However, the reduced form of tetrazine, dihydrotetrazine **3**, is formed in each of the adduct oxidation steps and was isolated from the reaction mixture. Furthermore, rearrangement of **3** to the aminotriazole **4** was also observed. When the reaction (utilizing **1a**) was carried out with triglyme as solvent, the aroma of benzonitrile (**5**) was detected and a swab of the condensate in the reflux condenser showed this substance to be identical to authentic benzonitrile by reverse-phase HPLC. The possibility that the imidazole ring resulted from the participation of benzonitrile (formed by the decomposition of **1a**) was ruled out by finding no reaction upon heating DBU with benzonitrile in triglyme at reflux. In addition, diphenyltetrazine (**1a**) was found to be stable in triglyme under

these reaction times (up to 3 h). These findings support the postulate of a seven member triazepine ring (**A**) being formed, which contracts with extrusion of the arylnitrile to deliver amino imidazole **B** (**Figure 3**).



3 4 5

Figure 3: Side-products from the reaction of **1** + DBU.

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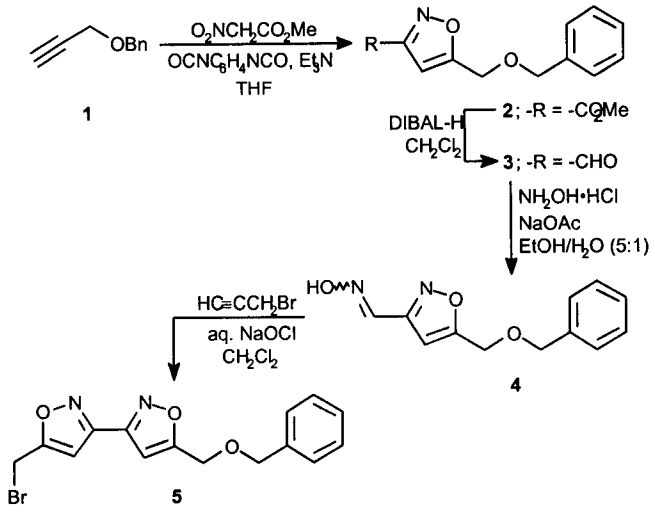
(2) **Synthesis of Linear Bidentate Diisoxazole and Bidentate Isoxazole-Furyl/Thienyl/Pyridyl Motifs**: The tetraheterocyclic synthesis commences with the preparation of benzyl propargyl ether (**1**) by the base-mediated (KH) coupling of benzyl alcohol and propargyl bromide. To avoid deprotonation of the terminal alkyne, benzyl alcohol was added first to the potassium hydride (1.0 equiv.) suspended in THF. After alkoxide formation was complete (30 min at 0 ℃), the electrophile was added. The benzyl propargyl ether was purified by vacuum distillation to give **1** (93%).

A modified Mukaiyama method, employing catalytic triethylamine and 1,4-phenylene diisocyanate instead of phenyl isocyanate, was used to dehydrate methyl nitroacetate generating the corresponding nitrile oxide *in situ*. Concomitant 1,3-dipolar cycloaddition to the alkyne moiety of **1** delivered isoxazole **2** in 76% yield (**Scheme 1**). This modified dehydration method results in the formation of a diphenyl urea polymer which is easily separated from the isoxazole product by filtration. The ethyl ester analog of isoxazole **2** (i.e., -R = $-CO_2Et$) was also prepared starting from the hydrochloride salt of glycine ethyl ester using a modified Huisgen cycloaddition method in 74% yield. In our hands, these two procedures for generating $RO_2C—C{\equiv}N^+—O^-$ were equally effective. Treating isoxazole **2** with DIBAL-H (1.2 equiv. in CH_2Cl_2 at -78 ℃) delivered aldehyde **3** in 96% yield.



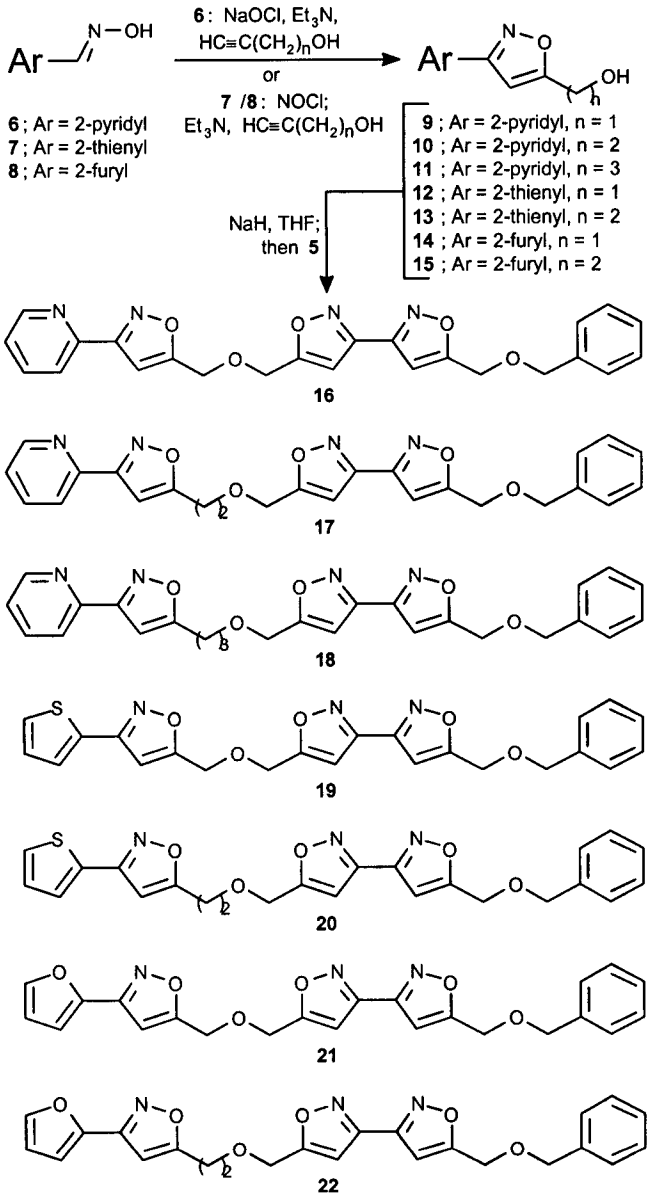
Scheme 1. Preparation of diheterocyclic intermediate **5**.

The oxime of aldehyde **3** was prepared using hydroxylamime hydrochloride and sodium acetate in a solution of ethanol-water. Isolation of this *E,Z*-oxime mixture (**4**; 99%) followed by addition to a CH_2Cl_2/aqueous sodium hypochlorite biphasic mixture containing propargyl bromide and catalytic triethylamine generated a nitrile oxide which underwent concomitant 1,3-dipolar cycloaddition. The oxime was dissolved in methylene chloride and added dropwise in an effort to reduce the formation of furoxan *via* nitrile oxide dimerization. The resulting 'head-to-head' C_3,$C_{3'}$-diisoxazole product **5** (80%) possesses the electrophilic -CH_2Br functional group necessary for the planned coupling.

Construction of the furyl/thienyl/pyridyl-isoxazole commenced with oxime formation (NH_2OH•HCl, NaOAc) from three 2-heterocarboxaldehydes giving 2-pyridinealdoxime (**6**), 2-thiophenealdoxime (**7**), and 2-furanaldoxime (**8**) in high yield (75-91%; **Scheme 2**). *In situ* nitrile oxide formation from **6** (aq. NaOCl) in the presence of either propargyl alcohol, 3-butyn-1-ol, or 4-pentyn-1-ol delivered 3-(2-pyridyl)isoxazoles **9-11** in good yield (66-77%). Some dimerized furoxan was observed (≤10% by crude NMR). Isoxazoles **9** has previously been examined for inhibition of thrombocyte aggregation and supercooperativity in platelet aggregation.

The use of aqueous sodium hypochlorite for the oxidation/dehydrochlorination of 2-thiophenealdoxime and 2-furanaldoxime was ineffective due to apparent anionic polymerization. It proved necessary to prepare nitrile oxides from **7** and **8** by a two step process—(1) halogenation followed by (2) dehydrohalogenation. The use of chlorine gas, NCS, and NBS all gave partial halogenation at the C_5 position of the thienyl and furyl heterocycles. Fortunately, nitrosyl chloride (NOCl) selectively oxidized the oxime moiety without effecting ring halogenation. And, having thus effected R-CH=N-OH → R-C(Cl)=N-OH, the appropriate alkynol was then added to the crude hydroximoyl chloride followed by slow addition of Et_3N by syringe pump. The isolated yields of cycloadducts **12-15** were in the 40-55% range. Based on regenerated aldoxime (Et_3N neutralization of unwanted aldoxime hydrochloride formed as a side product in the NOCl oxidation step) and starting 2-heterocarboxaldehyde (aldoxime hydrolysis), the yields for **7/8** → **12-15** were quite good (≈70%).

With fragments **5** and **9-15** in hand, we turned to the preparation of the tetraheterocyclic, triisoxazole-containing targets. This was accomplished in straightforward fashion by Williamson ether synthesis. One equivalent of both fragments were added to THF followed by introduction of base. Sodium hydride proved to by most expedient, giving *in situ* alkoxide formation and concomitant *O*-alkylation. A single product was obtained in excellent yield (80-90%) in the reaction of **5** with **9**, **11**, **12**, or **14**. However, in the case of 5-(2-hydroxyethyl)isoxazoles **10**, **13**, and **15**, some dehydration occurs giving a isoxazole-conjugated C_5-vinyl. In these reactions, the yields of **17**, **20**, and **22** were 72%, 75%, and 72%, respectively.



Scheme 2. Preparation of diheterocyclic intermediates **9-15** and coupling with **5**.

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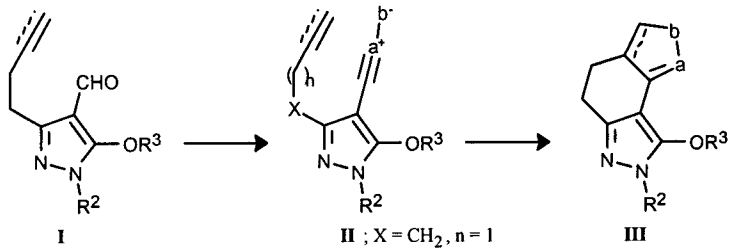
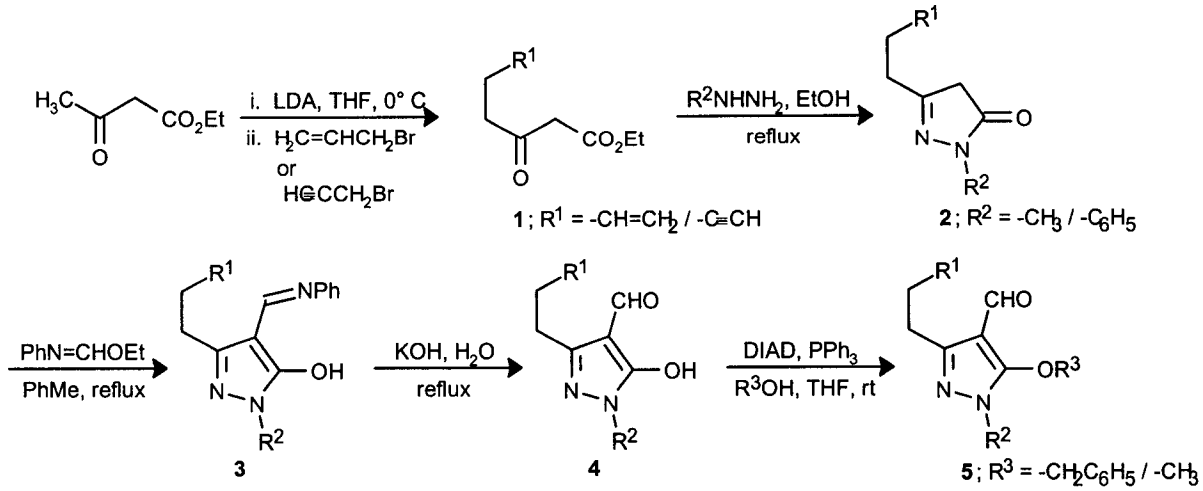
(3) **Synthesis of Pyrazolo[3,4-g][2,1]dihydrobenzoisoxazol(in)es**: We have explored the development of a general protocol for the preparation of these novel heterocycles from ethyl acetoacetate. In this paper, we report our initial studies on the construction of pyrazoloisoxazol(in)e-based heterocycle **III** utilizing the intramolecular cycloaddition of 1,3-dipole **II**, in turn generated from dipolarophile "tethered" pyrazolo aldehyde **I** (**Scheme 1**).



I II ; X = CH_2, n = 1 III

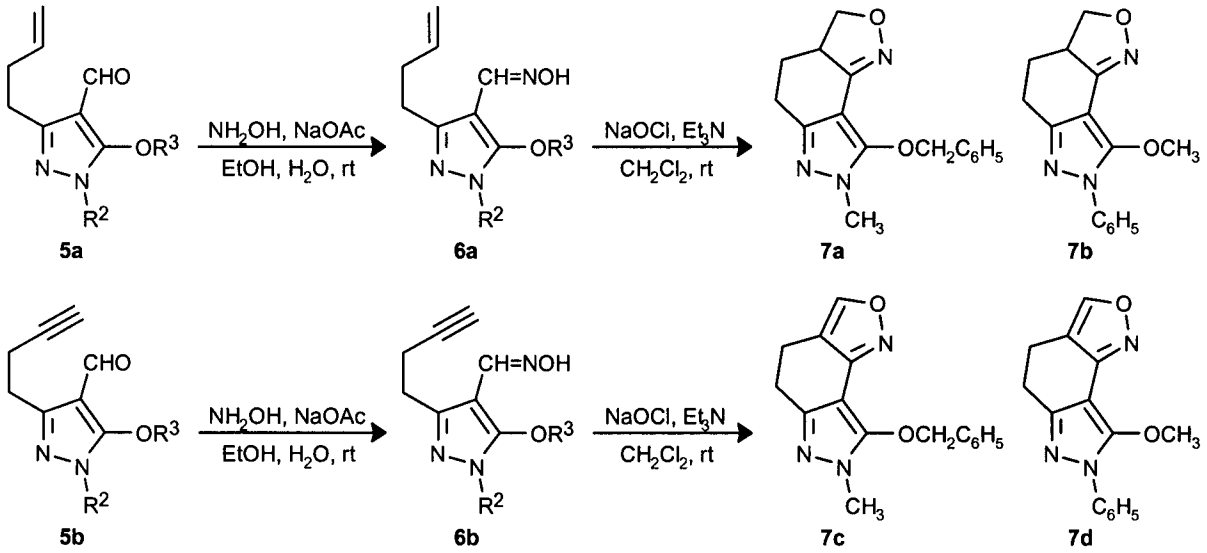
Scheme 1:

Our synthesis of the targeted heterocycles (**III**) begins as depicted in **Scheme 2**. Formation of the dianion of ethyl acetoacetate with 2 eq. of LDA at 0° C in THF followed by alkylation (propargyl or allyl bromide) provided β-keto ester **1** (R^1 = -C≡CH or -CH=CH_2) in 62% and 75% yield, respectively. Condensation of **1** with phenyl or methyl hydrazine in refluxing anhydrous ethanol gave the corresponding pyrazolone **2** (R^1 = -C≡CH or -CH=CH_2; R^2 = -C_6H_5 or -CH_3). Attempts to install a formyl group at C-4 using classic Vilsmeir conditions (DMF, $POCl_3$, 0° to 100° C, 2h) were not successful. Thus, introduction of the formyl group at C-4 was accomplished using a two step procedure involving formation of Schiff base **3** by condensing ethyl N-phenylformimidate with **2** in refluxing toluene. Subsequent hydrolysis of **3** with aqueous potassium hydroxide and neutralization with concentrated HCl provided 4-formyl derivative **4**. The presence of a C-5 hydroxyl tautomer in **4** was indicated by a broad O—H band at 2835 cm^{-1} in the infrared spectrum, a broad singlet at 10.12 ppm in the ^1H NMR spectrum, and ^{13}C NMR resonances at 104.9 and 158.4 ppm for C-4 and C-5, respectively. Model experiments revealed that it was necessary to mask the C-5 hydroxyl moiety prior to intramolecular nitrile oxide cycloaddition. Accordingly, **4** was alkylated under Mitsunobu conditions with methyl or benzyl alcohol to give pyrazole **5** (R^1 = -C≡CH or -CH=CH_2; R^2 = -C_6H_5 or -CH_3; R^3 = -CH_3 or -$CH_2C_6H_5$; in 32-40% yield from **4**).



Scheme 2:

With sufficient quantities of alkene (R^1 = -CH=CH_2) and alkyne (R^1 = -C∫CH) intermediates **5** in hand, we were in a position to prepare pyrazolo[3,4-g][2,1]-dihydrobenzoisoxazolines **7a/b** and pyrazolo[3,4-g][2,1]dihydrobenzoisoxazoles **7c/d** (**Scheme 3**). The requisite nitrile oxide intermediates were prepared in two steps by the Huisgen method. First, room temperature condensation of aldehyde **5** with hydroxylamine hydrochloride in 95% EtOH containing NaOAc (2.5 eq.) gave oxime **6**. Subsequent dropwise addition of aqueous sodium hypochlorite (5.25%) to a solution of this oxime and triethylamine in CH_2Cl_2 at 0° C generated the nitrile oxide intermediate which underwent concomitant cycloaddition to **7a-d** in 65-80% yield.



Scheme 3:

It is interesting to note that **7d** exhibited a four proton singlet at 2.85 ppm (methylene protons) in its $CDCl_3$ ^1H NMR spectrum. However, in benzene-d_6, these two methylenes were shifted to lower field and were rendered magnetically non-equivalent; appearing as two triplets centered at 2.02 and 2.33 ppm (J = 7.0 Hz). The isoxazole methine was also shifted upfield by nearly one ppm (from 8.11 ppm in $CDCl_3$ to 7.14 ppm in benzene-d_6). Compound **7c** behaves similarly in $CDCl_3$ and benzene-d_6.

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(4) **Synthesis of Spiro-Fused (C5)-Isoxazolino-(C4)-Pyrazolones**: The Baylis-Hillman reaction — a valuable carbon-carbon bond-forming reaction in organic chemistry — provides, in one pot, the α-anion equivalent of an electron deficient alkene. The electrophile is usually an aldehyde and the most common catalyst is DABCO (1,4-diazabicyclo[2.2.2]octane). In addition, Baylis-Hillman adducts have been widely utilized as intermediates in the synthesis of various target molecules. Visualizing these same intermediates in our approach to **C**, methyl acrylate could provide allylic alcohol adduct **I** (Figure 1). 1,3-Dipolar cycloaddition of nitrile oxides with this Baylis-Hillman adduct followed by oxidation should give ketone **H** regioselectively. We reasoned that treating this β-ketoester with monosubstituted hydrazines would deliver the

corresponding hydrazone which, by cycloelimination of methanol via an intramolecular hydrazone condensation with the ester, would provide the target isoxazolino-pyrazolones (**C**).

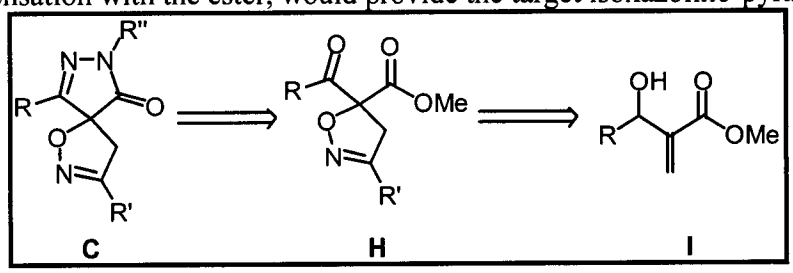
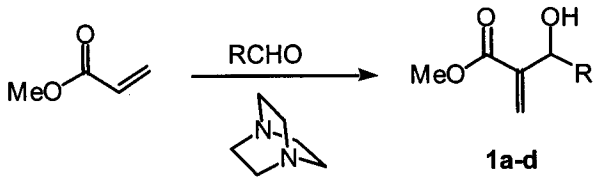


Figure 1: Retrosynthetic Approach to Isoxazolino-Pyrazolones

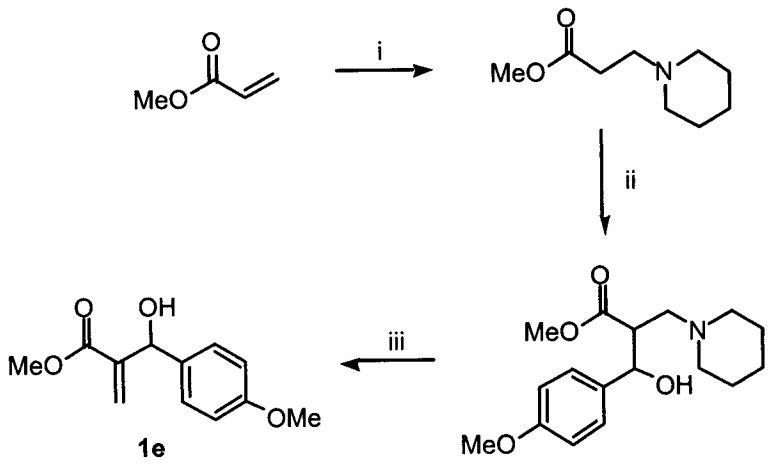
Our synthesis commenced with the Baylis-Hillman reaction between methyl acrylate and various aliphatic and aromatic aldehydes. Typical reports of the DABCO catalyzed Baylis-Hillman reaction require reaction times of several days to weeks, but the reaction is quite dependent on both the activated alkene and the aldehyde electrophile. As a result, many investigations into new catalysts and alternative reaction conditions have been attempted to expedite reaction times and increase yields. One account of the reaction between *tert*-butyl acrylate and benzaldehyde under normal Baylis-Hillman conditions provided the product in 90% yield, but the reaction took four weeks. Even more drastic cases are documented in the literature (33 and 62 days; 33, 38, 40, and 63 days; and 48 days). Despite these drawbacks and limitations of the Baylis-Hillman reaction, we were able to synthesize several allylic alcohols **1a-d** in acceptable yields using standard procedures (Scheme 1).



1a, R = Me, **1b**, R = Et; **1c**, R= Ph; **1d**, R = 4-MePh

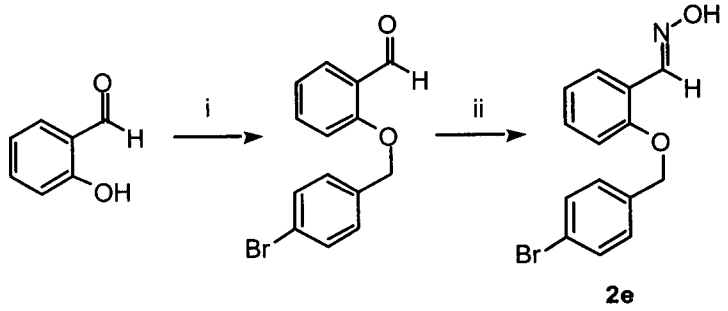
Scheme 1: Baylis-Hillman Reaction

Baylis-Hillman problems previously reported with *p*-anisaldehyde were confirmed in our hands. We found that a seldom used, step-wise, "MAC" procedure (Scheme 2) consisting of (i) Michael addition of an unhindered secondary amine (piperidine), (iia) lithium enolate formation of the β-aminoester with LDA, (iib) conversion to its zincate with $ZnCl_2$, (iic) Aldol reaction with various aldehydes, and (iii) *N*-oxide formation from the γ-aminoalcohol with MCPBA followed by concomitant Cope elimination allowed us to obtain Baylis-Hillman adduct **1e** the day after the process was initiated. While this work was in progress, an alternative protocol for the "stepwise" Baylis-Hillman reaction, which employs lithium phenylselenide, was published.



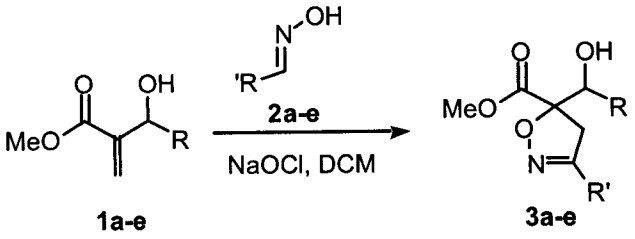
Scheme 2: Alternative MAC Route to Baylis-Hillman Adducts of *p*-Anisaldehyde: (i) Piperidine, THF, rt; (ii) (a) LDA, THF, -78 °C; (b) $ZnCl_2$, THF; (c) *p*-anisaldehyde; (iii) MCPBA, DCM, rt.

With the allylic alcohols (**1a-e**) in hand, we next investigated their 1,3-dipolar cycloaddition with various nitrile oxides. The requisite aldoximes **2a-d** were synthesized from the corresponding aldehydes by reaction with hydroxylamine, while aldoxime **2e** was prepared in two steps (Scheme 3) by *O*-alkylation of salicylaldehyde with 4-bromobenzyl bromide, sodium carbonate, and catalytic TBAI in DMF at 100 □C. This aldehyde was then converted into aldoxime **2e** by reaction with hydroxylamine.



Scheme 3: Synthesis of Aldoxime **2e** from Salicylaldehyde: (i) 4-BrBnBr, Na_2CO_3, TBAI, DMF, 100 °C; (ii) $H_2NON{\bullet}HCl$, NaOAc, THF/EtOH/H_2O

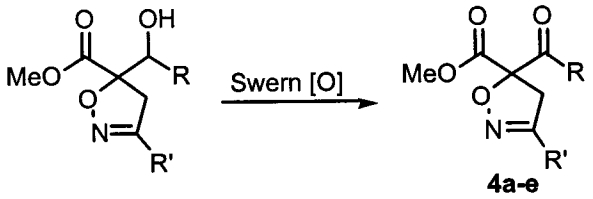
Employing bleach as oxidant converts oximes into nitrile oxides in situ and we selected this convenient, efficient method to generate nitrile oxides from aldoximes **2a-e**. The nitrile oxides were reacted with Baylis-Hillman adducts **1a-e** (**1a** with the nitrile oxide of **2a**, **1b** with **2b**, etc.) to give the corresponding isoxazolines **3a-e** (Scheme 4). Diastereoselectivity for these cycloadditions ranged from 1.4-2.6. In all cases, hydrogen bonding of the allylic alcohol with the nitrile oxide gives preference for the syn diastereomer; X-ray crystallographic analysis of the major diastereomer of **3e** confirms this diastereoselectivity. We generally did not separate the diastereomers or even purify these intermediates since the subsequent Swern oxidation (Scheme 5) removes the second stereocenter and proceeds efficiently on crude isoxazoline substrates. The structures and overall yields of these β-ketoesters are displayed in Table 1.



Scheme 4: 1,3-Dipolar Cycloaddition of Nitrile Oxides with Baylis-Hillman Adducts

3a-e and **4a-e**	R =	R' =	isolated yield of **4** from **1**
a	Me	2-ClPh	64%
b	Et	4-MeOPh	76%
c	Ph	2-Pyridyl	72%
d	4-MePh	2-MeOPh	73%
e	4-MeOPh	2-(4-BrBnO)Ph	66%

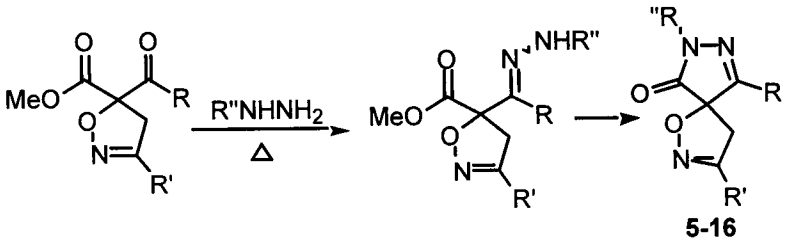
Table 1: Structures of Isoxazolines **3** and **4** and Overall Yields of **4**



Scheme 5: Swern Oxidation of Cycloadducts to Provide β-Ketoesters

Condensation of β-ketoesters **4a-e** with hydrazines was presumed to give the corresponding hydrazone that would then undergo cycloelimination to give the target isoxazolino-pyrazolones (Scheme 6, Table 2). We found, in all cases, that the final cycloelimination commenced before all starting β-ketoester was consumed. Indeed, hydrazone formation is difficult in most cases because of the steric hindrance of these ketones. The electron rich *p*-methoxyphenyl ketone further retarded the progress of this reaction and 27% of starting β-ketoester was isolated upon work-up and product purification. Fortunately, we found that TFA, or even better, $TiCl_4$, effectively catalyzed this conversion. Isomerization between the *E*- and *Z*-hydrazones must also be taken into account as only the *Z*-configuration is capable of cycloelimination. Unfortunately, the *Z*-isomer is sterically less favored as evidenced by X-ray

crystallographic analysis of the *E*-phenylhydrazone of **4c**; the corresponding *Z*-isomer was not detected.



Scheme 6: Hydrazone Formation and Cycloelimination to Afford Pyrazolones

	R	R'	R''	Isolated yield
5	Me	2-ClPh	Me	67
6	Me	2-ClPh	Ph	64
7	Et	4-MeOPh	Me	87
8	Et	4-MeOPh	Ph	72
9	Et	4-MeOPh	H	80
10	Et	4-MeOPh	Bn	77
11	Et	4-MeOPh	4-MeOPh	97
12	Ph	2-Pyridyl	Me	97
13	Ph	2-Pyridyl	Ph	44 (71)[a]
14	4-MePh	2-MeOPh	Me	72
15	4-MePh	2-MeOPh	Ph	58
16	4-MeOPh	2-(4-BrBnO)Ph	Me	45 (72)[b]

Table 2: Structure of Spiro Isoxazolino-pyrazolones **5-16** and Yields from **4a-e**: a) Yield based on isolated hydrazone. b) Yield based on isolated β-ketoester.

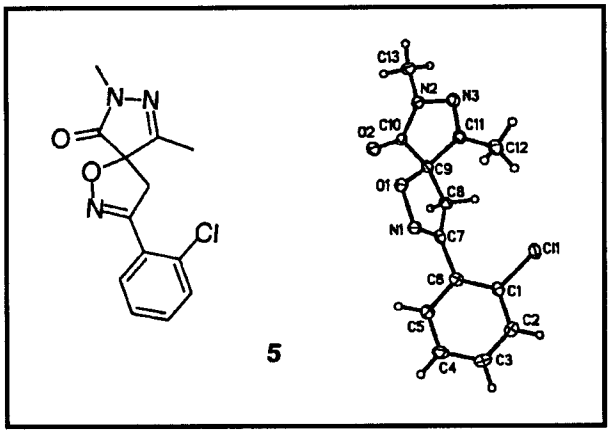


Figure 2: Computer Generated Single Crystal X-Ray Structure of **5**

Spiro-fused (C5)-isoxazolino-(C4)-pyrazolones are prepared by a four-step procedure from methyl acrylate consisting of a Baylis-Hillman reaction (or step-wise MAC procedure — Michael addition, Aldol reaction, and Cope elimination), 1,3-dipolar cycloaddition with nitrile oxides, Swern oxidation, and hydrazone formation with concomitant cycloelimination. With the exception of hydrazine itself, these condensations/cycloeliminations were difficult without catalysts or high temperature. $TiCl_4$ was an efficient catalyst for not only hydrazone formation, but also cycloelimination of methylhydrazine with β-ketoesters. Phenyl, benzyl, and 4-methoxyphenylhydrazine were determined to react more efficiently in refluxing xylene. The novel spiro-fused isoxazolino-pyrazolone (3,7,9-substituted-1-oxa-2,7,8-triaza-spiro[4.4]nona-2,8-dien-6-one) are constrcuted from three diversity inputs — RCHO, R'CH=NOH, and $R''NHNH_2$ — by a procedure which appears suitable for combinatorial library production.