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TITLE:

Solid-phase Synthesis of [4.4] Spirocyclic Oximes

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Solid-phase synthesis, regenerating Michael linker, intramolecular 1,3-dipolar cycloaddition, spirocyclic heterocycles, tricyclic intermediate, high diastereoselectivity

SUMMARY:

Here we present a protocol to demonstrate an efficient method for the synthesis of spirocyclic heterocycles. The five-step process utilizes solid-phase synthesis and regenerating Michael linker strategies. Generally difficult to synthesize, we present a customizable method for the synthesis of spirocyclic molecules otherwise inaccessible to other modern approaches.

ABSTRACT:

A convenient synthetic route for spirocyclic heterocycles is well sought after due to the molecule's potential use in biological systems. By means of solid-phase synthesis, regenerating Michael (REM) linker strategies, and 1,3-dipolar cycloaddition, a library of structurally similar heterocycles, both with and without a spirocyclic center, can be constructed. The main advantages of the solid-support synthesis are as follows: first, each reaction step can be driven to completion using a large excess of reagents resulting in high yields; next, the use of commercially available starting materials and reagents keep the costs low; finally, the reaction steps are easy to purify *via* simple filtration. The REM linker strategy is attractive because of its recyclability and traceless nature. Once a reaction scheme is completed, the linker can be reused multiple times. In a typical solid-phase synthesis, the product contains either a part of or the whole linker, which can prove undesirable. The REM linker is "traceless" and the point of attachment between the product and the polymer is indistinguishable. The high diastereoselectivity of the intramolecular 1,3-dipolar cycloaddition is well documented. Limited by the insolubility of the solid support, the reaction progression can only be monitored by a change in the functional groups (if any) *via* infrared (IR) spectroscopy. Thus, the structural identification of intermediates cannot be characterized by conventional nuclear magnetic resonance (NMR) spectroscopy. Other limitations to this method stem from the compatibilities

of the polymer/linker to the desired chemical reaction scheme. Herein we report a protocol that allows for the convenient production of spirocyclic heterocycles that, with simple modifications, can be automated with high-throughput techniques.

INTRODUCTION:

Despite recent discoveries using highly-functionalized spirocyclic heterocycles in a number of biological systems[1], a convenient pathway is still necessary for their easy manufacture. Such systems and uses for these heterocycles include: MDM2 inhibition and other anticancer activities[2-5], enzyme inhibition[6-8], antibiotic activity[9,10], fluorescent tagging[10-12], enantioselective binding for DNA probes[13-15] and RNA targeting[16], along with numerous potential applications to therapeutics[17-19]. With an increasing demand for these heterocycles, current literature remains divided about which synthetic pathway is best. Modern synthetic approaches to this problem use isatin and isatin derivatives as starting materials for a variety of heterocycles[20,21], complicated intramolecular rearrangements[22-25], Lewis acid[1,26,27] or transition metal catalysis[17,28-30], or asymmetric processes[31]. While these procedures have had success in producing specific spirocyclic oximes with limited functionality, a synthetic strategy for producing a library of molecules with high diastereoselectivity has been explored relatively less[32].

The technique presented here shows that these molecules of interest can be generated using a number of well-understood synthetic techniques in tandem. Starting with the synthesis of the molecule on a solid support using a REM linker and intramolecular silyl nitronate-olefin cycloaddition (ISOC), the proposed pathway deploys a nonlinear route, characterized by bond severing in a tricyclic system, leaving a highly functionalized heterocycle. REM linkers, known for their convenience and recyclability, utilize a solid support to synthesize tertiary amines[33]. Due to the ease of purification accredited to the REM linker *via* simple filtration, this solid-phase synthesis technique provides scientists with a recyclable and traceless linker, which has been used here. Once the reaction is complete, the REM linker is regenerated and can be reused multiple times. The REM linker is also traceless because, unlike many solid-phase linkers, the point of attachment between the product and the polymer is indistinguishable[34,35]. Also well-studied and understood is the ISOC reaction, useful in the synthesis of pyrrolidine oximes[36,37]. Perhaps better known as a 1,3-dipolar cycloaddition, these reactions form a number of heterocycles with high diastereoselectivity[38-45]. Using the modified REM-coupled-ISOC technique for the synthesis of spirocyclic molecules yields a highly diastereoselective product. Herein, we report on the efficient production of spirocyclic oximes using a new synthetic approach, combining two well-understood pathways and readily available starting materials.

PROTOCOL:

CAUTION: Please consult all relevant material safety data sheets (MSDS) before use. Several of the chemicals used in these syntheses are acutely toxic and carcinogenic. Please use all appropriate safety practices when performing the following reactions, including the use of engineering controls (fume hood and IR and NMR spectrometers) and personal protective equipment (safety goggles, gloves, lab coat, full-length pants, and closed-toe shoes).

1. Michael Addition of Furfurylamine to the REM Linker

NOTE: The duration of this step is 25 min for the set-up and 24 h of reaction time.

1.1. Add 1 g (1 equiv.) of REM resin, 20 mL (20 equiv.) of dimethylformamide (DMF), and 2.4 mL of furfurylamine to a 25 mL solid-phase reaction vessel.

1.2. Agitate the reaction vessel for 24 h at room temperature using a shaker following the reaction initiation. The vessel is capped during the reaction.

NOTE: Ensure that the resin does not sit at the bottom of the vessel and mixes thoroughly.

1.3. Drain the solution and wash the resin 1x with 5 mL of DMF after the reaction is complete.

1.3.1. Then, wash the resin 4x, alternating between 5 mL of dichloromethane (DCM) and 5 mL of methanol.

1.3.2. Following the washes, dry the resin thoroughly with compressed air in the reaction vessel for 30 min.

1.3.3. Monitor the reaction progress for a change in IR stretching frequencies, as shown in **Table 1**.

2. Tandem Michael Addition/1,3-dipolar Cycloaddition

NOTE: The duration of this step is 25 min for the set-up and 48 h of reaction time.

2.1. Take the dry resin and add 1.48 mL (5 equiv.) of triethylamine (TEA), 10 mL of dry toluene, and 0.637 g (2 equiv.) of nitro-olefin to the reaction vessel.

2.2. Add 1 mL (4 equiv.) of trimethylsilyl chloride (TMSCl) to the reaction vessel in a well-ventilated fume hood.

CAUTION: This reaction will form HCl gas. Do not cap the reaction vessel until the gas has been released under a fume hood.

2.3. Securely cap the reaction vessel and agitate using a shaker for 48 h at room temperature. Ensure that the resin mixes thoroughly with the reagents.

2.4. Quench the reaction with 5 mL of methanol.

2.4.1. Drain the solution from the vessel and, then, wash the resin 4x, alternating between 5 mL of DCM and 5 mL of methanol.

2.4.2. Following the washes, dry the resin thoroughly with compressed air in the reaction vessel for 30 min.

2.4.3. Monitor the reaction progress by observing a change in the IR stretching frequencies, as shown in **Table 1**.

3. Ring Opening of Resin-bound Isoxazole by Tetra-n-butylammonium Fluoride

NOTE: The duration of this step is 10 min for the set-up and 12 h of reaction time.

3.1. Place 1 mL of dry tetrahydrofuran (THF) in the reaction vessel with the dry resin. Then, add 1.24 mL (2 equiv.) of 1 M tetra-n-butylammonium fluoride (TBAF) in THF to the reaction vessel.

3.2. Using a shaker, agitate the solution for 12 h at room temperature and ensure that the resin thoroughly mixes with the solution.

3.3. Drain the solution and wash the resin 1x with 5 mL of THF after the reaction is complete.

3.3.1. Then, wash the resin 4x, alternating between 5 mL of DCM and 5 mL of methanol.

3.3.2. Following the washes, dry the resin thoroughly with compressed air in the reaction vessel for 30 min.

3.3.3. Monitor the reaction progress by observing a change in the IR stretching frequencies, as shown in **Table 1**.

4. *N*-alkylation of the Resin-bound Heterocycle to Form Quaternary Amine

NOTE: The duration of this step is 10 min for the set-up and 24 h of reaction time.

4.1. Take the dry resin in the reaction vessel and add 5 mL of DMF.

4.1.1. Then, add 1 mL of alkyl halide (10 equiv.) to the vessel and agitate using a shaker for 24 h at room temperature. Ensure the thorough mixing of the resin with the reagents.

4.2. Drain the solution and wash the resin 1x with 5 mL of DMF after the reaction is complete.

4.2.1. Then, wash the resin 4x, alternating between 5 mL of DCM and 5 mL of methanol.

4.2.2. Following the washes, dry the resin thoroughly with compressed air in the reaction vessel for 30 min.

4.2.3. Monitor the reaction progress by observing a change in IR stretching frequencies as shown in **Table 1**.

5. β-elimination of the Quaternary Amine from the Polymer Support

NOTE: The duration of this step is 15 min for the set-up and 24 h of reaction time.

5.1. Take the dry resin and add 3 mL of DCM to the reaction vessel.

5.1.1. Then, add 1.5 mL (5 equiv.) of TEA to the reaction vessel to cleave the heterocycle from the polymer support.

5.1.2. Agitate using a shaker for 24 h, ensuring the thorough mixing of the resin with the solution. Drain the solution from the resin.

NOTE: Do not discard since the cleaved product is in the TEA/DCM solution.

5.2. Wash the resin 4x, alternating between 5 mL of DCM and 5 mL of methanol.

NOTE: Do not discard.

5.2.1. Combine the elution from all washes in steps 5.1.2 and 5.2 and concentrate it *via* rotatory evaporation.

5.2.2. Purify the spirocyclic oxime by trituration: add 0.5 mL of hot methanol to dissolve any impurities. The pure product will crash out of the solution and is collected *via* gravity filtration.

5.3. Following two washes with 5 mL of DCM for reuse in future experiments, thoroughly dry the resin with compressed air in the reaction vessel for 30 min.

5.3.1. Monitor the reaction progress by observing a change in the IR stretching frequencies, as shown in **Table 1**.

REPRESENTATIVE RESULTS:

As outlined in the procedure above, the synthetic route to spirocyclic oximes (see **Figure 1**) begins with the Michael addition of furfurylamine to compound **1**, the REM linker, to afford **2**. A subsequent Michael addition and 1,3-dipolar cycloaddition of the support **2** using various β-nitrostyrene derivatives yield the tricyclic compound **3**, an *N*-silyloxy isoxazolidine with four unique stereogenic centers. Desilylation of **3** with TBAF produces the spirocyclic oxime **4**, still bound to the solid-phase linker. Following the desilylation of **3**, polymer-bound **4** is *N*-alkylated with various electrophiles of choice yielding an ammonium salt, as seen with compound **5**. Finally, using β-elimination for the cleavage from the polymer support, compound **6** is generated, along with the fully intact REM linker **1**. A library of spirocyclic molecules can be created and purified with ease based on the choice of R_1, the β-nitrostyrene, and R_2, the electrophiles used in *N*-alkylation.

For monitoring the progress of each reaction step shown in **Figure 1**, IR spectroscopy was done on the starting REM resin **1** and on each of the polymer-bound intermediates **2** - **5** to determine whether or not each step had proceeded to completion. These could be classified with a change in the functional group, including conjugated or unconjugated esters, trimethylsilyls, hydroxyls, and oximes, corresponding to a change in wavenumbers as shown in **Table 1**. NMR analysis was not used to monitor the progress of each step since the intermediates formed are bound to the insoluble polymer support. Corresponding diastereoselective ratios (dr) and yields of the six products **6a** - **6f** are depicted in **Table 2**. The yields between 40% and 53% are the overall yields which highlight an average, high yield of between 80% and 88% per step in this five-step route. ^{1}H NMR analysis of the crude product mixture provided the dr values reported.

FIGURE AND TABLE LEGENDS:

Figure 1: REM-coupled-ISOC technique for the synthesis of spirocyclic oximes through a tricyclic system intermediate. Customizable R_1 and R_2 groups using commercially available β-nitrostyrene derivatives and different alkylating reagents, respectively, allow for a library of molecules with a common, spirocyclic backbone to be created, as shown in molecule **6**.

Table 1: Monitoring solid-phase reactions by infrared spectroscopy. A reaction progression determination of each step was conducted by tracking the changes in the IR stretching frequencies of the starting REM resin **1** and the intermediates **2** - **5**.

Table 2: Solid-phase synthesis of *N*-octyl, -methyl, -allyl, and -benzyl, spirocyclic oximes (products 6a - 6f). (**a**) The diastereoselective ratio was determined by ^{1}H NMR spectroscopy. (**b**) The reported yield of the five-step synthesis was determined based on the loading of the REM resin. The overall yield of 40% - 53% indicates an average of 80% - 88% yield for each step.

DISCUSSION:

In a typical REM linker/solid-phase synthetic strategy, prior to the release of an amine from the solid support, it is critical to form a quaternary ammonium salt, as described in section 4 of the protocol[39]. Due to the steric hindrance of the tricyclic system and bulky R_2 groups (benzyl and octyl halides), only small alkylating reagents (methyl and allyl halides) could be utilized in this reaction[46]. With a simple modification, allowing for the addition and use of larger, steric reagents, the rigidity of the tricyclic structure was decreased before the *N*-alkylation step by opening the isoxazoline ring first[32]. This is illustrated in **Figure 1**. The ring opening of the tricyclic intermediate **3** relieves the steric hindrance which allows for the addition of virtually any primary alkyl halide desired.

This method was successful in reporting some of the highest dr values in the synthesis of spirocyclic compounds[30,47,48]. Success in the diastereoselectivity is attributed to the ISOC reaction, which takes the furfurylamine moiety of **2** and creates the rigid, tricyclic system of **3**[38-40]. Further steps, such as the breaking of the tricyclic system, conserve the diastereoselective nature of the molecule, affording the scientist, in the end, with compounds at or above

diastereoselective ratios of 95:5. Equally important is the customizability of the method: with modified β-nitrostyrene derivatives and other electrophiles for *N*-alkylation, a large library of molecules can be made with relative ease.

To conclude, a highly diastereoselective protocol for the construction of highly functionalized, spirocyclic molecules using a new REM-coupled-ISOC pathway has been developed. This pathway yields a rigid, tricyclic scaffold from the ISOC reaction, from which diastereoselectivity is conserved throughout the remaining reactions. The availability of β-nitrostyrene derivatives and alkylating reagents makes the route convenient and cost-effective. However, should they not be available for purchase, the synthesis of such reagents would be required. This is one such limitation of the method, another being the size of the cycles. As of now, the proposed method is suitable for the construction of a [4.4] spirocyclic framework. Limitations in the 1,3-dipolar cycloaddition method prevent the formation of other ring sizes.

We are in the process of testing the recyclability of the REM linker used in the protocol presented here and will report this shortly. In addition, future applications of the proposed method will be put to use in a number of biological assays. A high-throughput combinatorial synthesis of these spirocyclic molecules using this method can afford a large number of spirocyclic derivatives, which can be tested for anticancer activities in human cancer cells. Such tests will involve cytotoxicity assays, pull-down experiments, and cell culture viability.

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DISCLOSURES:

The authors have nothing to disclose.

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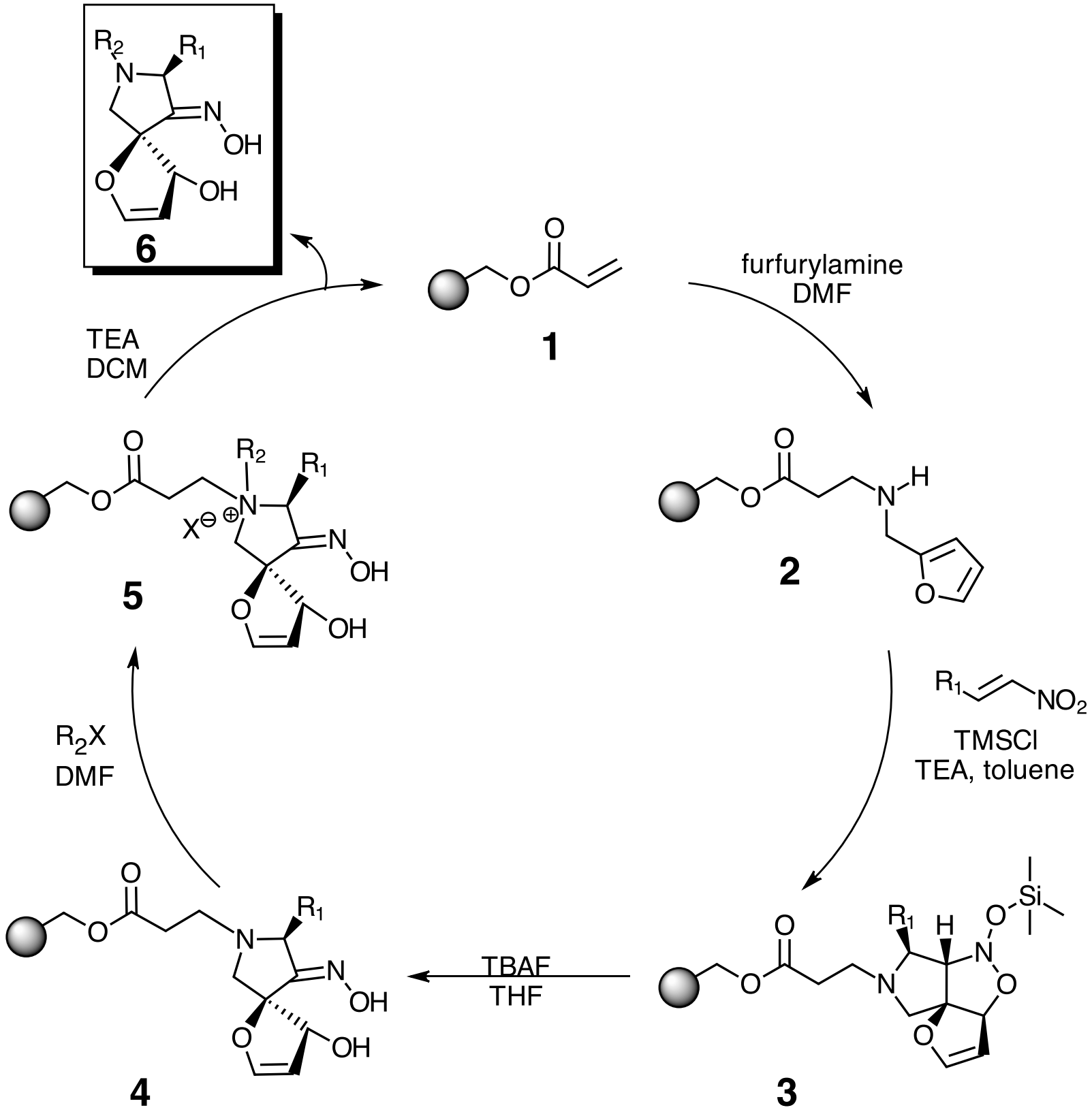

Starting material and intermediate	IR stretching frequency (cm^{-1})	IR Detectable Functional Groups
1	1722	Conjugated Ester
2	1731	Unconjugated Ester
3	1731	Unconjugated Ester
	1214	Trimethylsilyl
4	3600	Hydroxyl
	1731	Unconjugated Ester
	1655	Oxime
5	3600	Hydroxyl
	1731	Unconjugated Ester
	1655	Oxime

Product	R_1	R_2	dr[a]	yield (%)[b]
6a	phenyl	octyl	>99:1	40%
6b	phenyl	methyl	95:5	50%
6c	4-bromophenyl	methyl	96:4	53%
6d	4-bromophenyl	allyl	96:4	45%
6e	3,4-dimethoxyphenyl	benzyl	97:3	45%
6f	2,4-dichlorophenyl	methyl	>99:1	40%

Table of Materials

Name of Material/ Equipment	Company	Catalog Number
Chemicals		
REM Resin	Nova Biochem	8551010005
Furfurylamine	Acros Organics	119800050
Dimethylformamide (DMF)	Sigma-Aldrich	227056
Dichloromethane (DCM)	Sigma-Aldrich	270997
Methanol	Sigma-Aldrich	34860
trans -4-bromo-β-nitrostyrene	Sigma-Aldrich	400017
trans -3,4-dimethoxy-β-nitrostyrene	Sigma-Aldrich	S752215
trans -2,4-dichloro-β-nitrostyrene	Sigma-Aldrich	642169
trans -β-nitrostyrene	Sigma-Aldrich	N26806
Triethylamine (TEA)	Sigma-Aldrich	T0886
Trimethylsilyl chloride (TMSCl)	Sigma-Aldrich	386529
Tetra-n-butylammonium fluoride (TBAF) in Tetrahydrofuran (THF)	Sigma-Aldrich	216143
Tetrahydrofuran (THF)	Sigma-Aldrich	401757
1-Bromooctane	Sigma-Aldrich	152951
Iodomethane	Sigma-Aldrich	289566
Allylbromide	Sigma-Aldrich	337528
Benzylbromide	Sigma-Aldrich	B17905
Glassware/Instrumentation		
25 mL solid-phase reaction vessel	Chemglass	CG-1861-02
Thermo Scientific Nicole iS5	Thermo Scientific	IQLAADGAAGFAHDMAZA
AVANCE III NMR Spectrometer	Bruker	N/A
Wrist-Action Shaker Model 75	Burrell Scientific	757950819

	Comments/Description
	Solid Polymer Support; 1.1 mmol/g loading
	Reagent
	Solvent
	Solvent
	Solvent
	Nitro-olefin solid
	Nitro-olefin solid
	Nitro-olefin solid
	Nitro-olefin solid
	Solvent
	Reagent; CAUTION - highly volatile; creates HCl gas
	Reagent
	Reagent
	Alkyl-halide
	Alkyl-halide
	Alkyl-halide
	Alkyl-halide
	Glassware with filter
	Instrument
	Instrument; 300 MHz; Solvents: $CDCl_3$ and CD_3OH
	Instrument

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Author(s): Cody R. Drisko, Silas A. Griffin, and Kevin S. Huang

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Rebuttal Document

Please find below our rebuttal that addresses each of the editorial and peer review comments. Our rebuttal/address are in ***BLUE***, editorial's comments in **BLACK**, and the reviewer's in **RED**.

Editorial comments:

1. **Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.**

We have proofread to ensure fidelity in spelling and grammar in our submission.

2. **Please note that Open Access is checked in the uploaded ALA, while in the Questionnaire Responses Standard Access is selected. Please be consistent.**

We will be submitting our work as "Standard Access."

3. **Keywords: Please provide at least 6 keywords or phrases.**

We have added "Tricyclic Intermediate" and "High Diastereoselectivity" in line 19 to fulfill the 6 keywords or phrases requirement

4. **Please rephrase the Short Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to …"**

We have added the following in the SHORT ABSTRACT section (lines 22-25) that fulfills the 10-50 words limit. "Here we present a protocol to demonstrate an efficient method for the synthesis of spirocyclic heterocycles. The five-step process utilizes solid-phase synthesis and regenerating Michael linker strategies. Generally difficult to synthesize, we present a customizable method for the synthesis of spirocyclic molecules otherwise inaccessible to other modern approaches."

5. **Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.**

We have updated all the SI abbreviation units as seen in lines as seen in the PROTOCOL section in lines 100-242 (Revised).

6. **Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.**

We have updated all the spacing between all numbers and the corresponding units as seen in the PROTOCOL section in lines 100-242 (Revised).

7. **1.2: What is used to agitate the reaction vessel? Is the vessel capped during reaction?**

We have specified the use of the Burrell Wrist Action Shaker Model for agitation as seen in lines (Revised) 106, 132, 166, 184-5, and 226.

8. **1.3, 3.3, 4.2: Is solution drained after reaction is complete and before wash? Please specify.**

We have specified the solution drained after the reaction is completed in 1.3 (line 111, Revised), 3.3 (line 169, Revised), and 4.2 (line 189, Revised).

9. **1.3.2: Please mention how long it takes to dry the resin. Please specify throughout.**

We have specified the 30 min time requirement to dry the resin thoroughly with compressed air as seen in lines (Revised) 154, 173, 193, and 241.

10. **5.2.1: Please specify the elution from all washes. Does it mean the elution from each wash in step 5.2? Is elution from each step combined?**

The elution is from each wash in step 5.2 and are combined. We clarified this in lines 231-236 (Revised).

"5.2. Wash 4x alternating between 5 mL of DCM and 5 mL of methanol.
Note: Do not discard.
5.2.1. Combine the elution from all washes in step 5.1.2 and 5.2 and concentrate via rotatory evaporation."

11. **5.2.2: Please add more details to your protocol step. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.**

We have provided specific instructions on how we perform the trituration step as seen in lines (Revised) 238-239.

"5.2.2. Purify spirocyclic oxime by trituration: add 0.5 mL of hot methanol to dissolve any impurities. The pure product will crash out of the solution and is collected via gravity filtration."

12. **5.3: What is used to wash the resin and how many times?**

We have clarified this in line 241 (Revised).

"5.3. Dry the resin with compressed air thoroughly for 30 min in reaction vessel following the two washes with 5 mL of DCM for reuse in future experiments."

13. **Please consider including reaction progress monitoring of each step by IR spectroscopy and 1H NMR analysis in the protocol.**

IR SPECTROSCOPY. We have included monitoring the reaction progress by IR spectroscopy in lines 276-280 (Revised). For monitoring the progress of each reaction step shown in figure 1, infrared (IR) spectroscopy was done on the starting REM resin 1 and each of the polymer bound intermediates 2-5 to determine whether or not each step had proceeded to completion. These could be

classified with a change in functional group, including conjugated or unconjugated esters, trimethylsilyl, hydroxyls, and oximes, corresponding to a change in wavenumbers as shown in table 1.

NMR SPECTROSCOPY. Since the reaction is done on an insoluble polymer matrix, 1H NMR was not used to monitor the progress of each reaction step. We have stated this in lines 280-282 (Revised). "NMR analysis was not used to monitor the progress of each step since the intermediates formed are bound to the insoluble polymer support."

14. **Table 1 showed five compounds while Table 2 showed six. Please clarify or revise to be consistent.**

Table 1 shows the detectable IR stretching frequency of the polymer bound REM starting material 1 and intermediates 2-5. These stretching frequency numbers are used to monitor the progress each reaction step as seen in Figure 1. Table 2 shows the overall % yield and diastereoselective ratios of the desired products 6 obtained from the reaction scheme. Thus, a total of six different spirocyclic compounds (6a-6f) with different R_1 and R_2 substituents were obtained from this methodology. We believe this is clarified in lines 276-285.

"For monitoring the progress of each reaction step shown in figure 1, infrared (IR) spectroscopy was done on the starting REM resin 1 and each of the polymer bound intermediates 2-5 to determine whether or not each step had proceeded to completion. These could be classified with a change in functional group, including conjugated or unconjugated esters, trimethylsilyl, hydroxyls, and oximes, corresponding to a change in wavenumbers as shown in table 1. NMR analysis was not used to monitor the progress of each step since the intermediates formed are bound to the insoluble polymer support. Corresponding diastereoselective ratios (dr) and yields of the six products 6a-f are depicted in table 2. The yields between 40 and 53% are the overall yields which highlight an average, high yield of between 80 and 88% per step in this five-step route. ^{1}H NMR analysis of the crude product mixture provided the dr values reported."

15. **As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:**
    a) Critical steps within the protocol

We have clarified the critical step in our synthesis in line 306-307 (Revised).

b) Any modifications and troubleshooting of the technique

We have stated the modification/troubling shooting made in lines 307-313 (Revised).

c) Any limitations of the technique

We have stated the limitations in lines361-365 (Revised).

d) The significance with respect to existing methods

We have stated the significance in lines 315-355 (Revised).

e) Any future applications of the technique

We have stated the future applications of the techniques in lines 367-372 (Revised), specifically in testing the recyclability of the used linker and in using this technique in a high-throughput combinatorial synthesis of these spirocyclic compounds.

16. **Please submit each figure as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 pixels x 1080 pixels or 300 dpi.**

We will be submitting Figure 1 as an .eps format.

Reviewer #1:

Manuscript Summary: Authors describe an efficient five-step synthesis of spirocyclic multifunctional derivatives, using solid-phase synthesis and diversity building from b-nitrostyrenes and alkyl halides. Overall yields of 40-53% are noteworthy for such a multistep procedure. Experimental write-up is more or less straightforward for scientists to read.

Major Concerns: none

Minor Concerns:

1. **There are a couple of typos and grammatical errors but it seems like in-house copy-editing services can take care of these.**

We have proofread to ensure fidelity in spelling and grammar in our submission.

2. **Lines 102,104,126,139,141,155,157,171,175 - might be worthy to specify amounts used. Please include a space between all numbers and their corresponding units.**

Because of the changes in the text, the lines are now different. We have included the amounts in line 102 (111, Revised), 104 (114, Revised), 126 (139, Revised), 139 (169, Revised), 141 (171, Revised), 155 (184, Revised), 157 (191, Revised), 171 (231, Revised), and 175 (238, Revised). The space between all numbers and their corresponding units have been addressed as well.

3. **Lines 106,128,143,159,177 - what is an implied procedure for drying the resin?**

We have clarified the procedure for drying the resin as stated in lines 106 (now 115), 128 (154, Revised), 143 (173, Revised), 159 (193, Revised), and 177 (241, Revised): "Dry resin thoroughly with compressed air for 30 min in reaction vessel following washes"

4. **Line 36 - while regenerative Michael linker procedure is claimed, it is recommended to compare yield (for example, for 6a) by running the procedure using a recycled resin.**

Since we have not compared yields by running the procedure using a recycled REM resin, we added the following in lines 353-354 (Revised) "We are in the process of testing the recyclability of the REM linker in our protocol and will report this shortly." In addition, we removed the word "Recyclable" in the title, line 25 (Original)."

5. **How was relative stereochemistry determined for compounds 3-5? For instance, how was it established that R1, tertiary H on a neighboring carbon, and the vinyl ether piece are all pointing the same direction?**

The relative stereochemical determination was based on the x-ray data from our previous tetrahedron paper (Reference #32, line 475, Revised).

Reviewer #3:

- Are the title and abstract appropriate for this methods article? **Title … I would suggest replacing "spirocyclic oximes" in the title with "1-oxa-7-azaspiro[4.4]nonane"**

Though the "1-oxa-7-azaspiro[4.4]nonane" is the IUPAC name of these interesting heterocycles, we feel that this name might be too technical and thus would like to keep "spirocyclic oxime" in the title if possible.

- Abstract … **no suggested changes**
- Are there any other potential applications for the method/protocol the authors could discuss? **I have no additional suggested applications.**
- Are all the materials and equipment needed listed in the table? (Please note that any basic lab materials or equipment do not need to be listed, e.g. pipettes.) **No, I find the list to be complete.**
- Do you think the steps listed in the procedure would lead to the described outcome? **Yes; the description is well presented and the outcome is clearly as described.**
- Are the steps listed in the procedure clearly explained? **Yes; also the simplicity of the protocol is well presented.**
- Are any important steps missing from the procedure?

We have thoroughly look over the procedure to make sure important steps are not missing.

- **Volumes used in the washing steps would be useful (currently not provided).**

We have included all volumes used in the washing steps in lines (Revised) 112, 114, 140, 170, 172, 190, 192, 232, and 243.

- Are appropriate controls suggested? **Yes; the protocols are well described.**
- Are all the critical steps highlighted? **Yes.**
- Is there any additional information that would be useful to include?

1) I find lines 226-228 in DISCUSSION to be oddly presented … this sentence needs to be re-written for clarity.

We have re-worded lines 226-228 (313-315 in the Revised) as "This is illustrated in figure 1. Ring opening of the tricyclic intermediate 3 relieves the steric hindrance which allows for the addition of virtually any primary alkyl halide desired."

2) "linear support and creates a rigid, tricyclic system" in line 232 is an odd statement; I would find "furfurylamine moiety of 2 and creates the rigid, tricyclic heterocycle of 3" to be a much clearer statement.

We agree with the reviewer's comments and welcome his suggested word changes (line 317, Revised)

- Are the anticipated results reasonable, and if so, are they useful to readers? **Yes. The authors present a very interesting and, I believe, useful method for the stereo controlled synthesis of highly functionalized compound 6.**
- Are any important references missing and are the included references useful? **The referencing is fine … indeed, very thorough.**
- **I am pleased to recommend this work for JOVE publication.**

We are grateful for the reviewer's recommendation for publication!