**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.***

1. **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.”***

1. **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***

1. **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.”***

1. **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).***

1. **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).***

1. **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.***

1. **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).***

1. **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.***

1. **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.”**

1. **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.”***

1. **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.”***

1. **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.”***

1. **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 R1 and R2 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. 1H NMR analysis of the crude product mixture provided the dr values reported.”***

1. **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.***

1. **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.***

1. **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.***

1. **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”***

1. **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).”***

1. **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!***