

Expanding on the Purification Methodology of Polyethylene Glycol (PEG) Bound Molecules: The Synthesis of 3,5-Pyrazolidinediones

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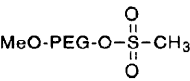
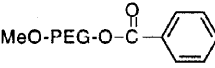
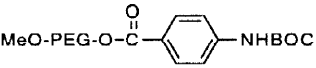
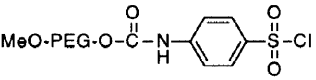
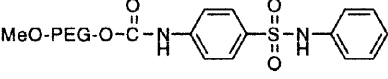
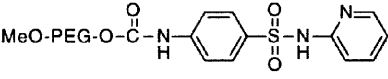
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Abstract: Isopropanol was found to be an excellent solvent for the precipitation of PEG-bound molecules. Polar reagents and by-products, even those generated as a result of using benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) or benzotriazol-1-yloxy-tripyrrolidinophosphonium hexafluorophosphate (PyBOP) coupling reagents, are soluble in iPrOH allowing clean precipitation of the polymer-bound species. Several examples are given to support the general utility of this precipitation method which allowed for the synthesis of a 3,5-pyrazolidinedione on soluble PEG polymer support.

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When polar reagents and/or polar by-products are produced in a polymer reaction, precipitation with Et₂O and subsequent purification can be troublesome. This problem is a limiting factor in many reactions performed on a soluble polymer support. To develop an efficacious means for the workup and purification of the polymer-bound species, it is crucial that a solvent be used that solubilizes the polar reagents and byproducts but precipitates the polymer-bound products. Based on previous work in our laboratory¹, generated PEG mesylate **1** (table 1), can easily be purified by removing the Et₃N·HCl byproduct with iPrOH. Therefore direct precipitation with iPrOH instead of Et₂O should solublize other polar reagents and byproducts and cleanly precipitate the polymer-bound product. Further investigation found that the use of iPrOH as the precipitating solvent can be applied in general for PEG-bound reactions regardless of reagents and reaction conditions. PEG-bound esters **2,3** and arylsulfonamides² **4-6** were prepared and precipitated from isopropanol as shown in Table 1. Release of the substrate from the polymer provided clean product, without need for further purification. We employed this precipitation method for the synthesis of a 3,5-pyrazolidinedione on the soluble PEG polymer support.

Table 1

Compd. Number	Polymer-bound Substrate	%Yield
1		95
2		95
3		93
4		97
5		92
6		92

3,5-Pyrazolidinediones **7** are a class of five-membered nitrogen-containing heterocyclic compounds (figure 1), which have been used clinically for the treatment of rheumatoid arthritis and various other diseases.³ The major side effect of these agents is gastric irritation which sometimes limits their therapeutic application. Since the biological activity of this series of compounds is of interest, a method of rapidly preparing analogues which may provide compounds with increased activity and diminished side effects would be advantageous. Within the scaffold of **7** two possible tautomers exist.⁴ However, if there are two substituents at the 4-position, there should be no tautomerization. Introduction of a second substituent will also result in structural changes to the molecule regarding its shape and size. More recent studies with this class of compounds have shown that disubstitution at the 4-position does indeed change their biological profile.⁵ Therefore, we wish to report our preliminary results on the synthesis of a 3,5-pyrazolidinedione of structure type **8** (figure 1) prepared on soluble polymer (PEG), and on the use of isopropanol for the precipitation of PEG-bound molecules.

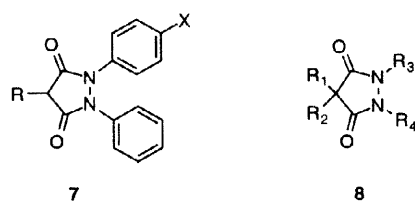
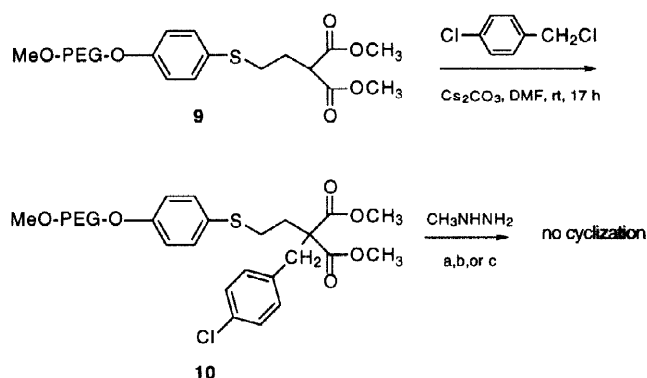


Figure 1.

With a novel synthetic strategy for the incorporation of malonate bound to PEG already reported by our laboratory⁶ and a new solvent system (MeOH/DMF, 1:8) reported for the traceless desulfonylation of less reactive polymer-bound aryl alkyl sulfones⁷, the synthesis of 3,5-pyrazolidinediones on PEG polymer support⁸ and subsequent detachment from the polymer could easily be envisioned. The key step in the synthesis is the coupling reaction of the substituted hydrazine with the polymer tethered dialkylated malonate. Unfortunately, when methylhydrazine was used for the cyclization reaction, under various reaction conditions, none of the desired product was obtained (Scheme 1).

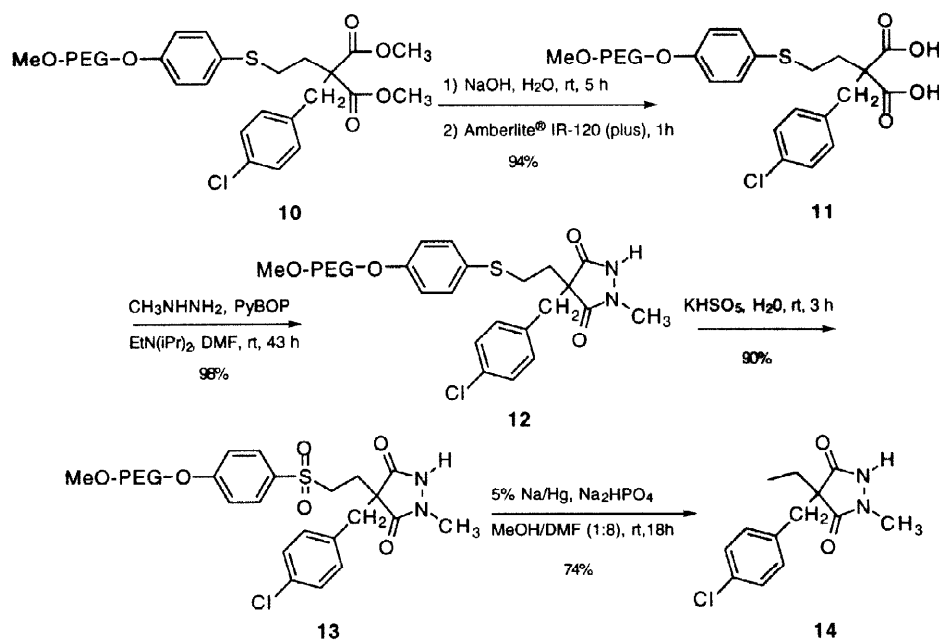


Scheme 1. a) absolute MeOH, reflux, 5 h b) Cs_2CO_3 , DMF, 100 °C, 15 h c) NaH, DMF, rt, 17 h

Since BOP and PyBOP are highly efficient coupling reagents, the cyclization might be accomplished by the reaction of the polymer-bound dialkylated malonic acid with methylhydrazine in the presence of BOP or PyBOP. Thus, the dialkylated malonate **10** was subjected to hydrolysis with NaOH/ H_2O to give **11** (Scheme 2).

The workup was facilitated with Amberlite® IR-120(plus). The BOP-mediated coupling reaction of **11** with methylhydrazine was then carried out. Unexpectedly, when the standard purification protocol using diethylether (Et₂O) was engaged, an amorphous solid was obtained instead of a normal crystalline polymer-bound product. Subsequently filtration of this material was extremely difficult, and all attempts to crystallize the polymer-bound product failed.

When polymer bound dialkylated malonic acid was reacted with methylhydrazine using BOP or PyBOP, precipitation with iPrOH gave excellent yields of 3,5-pyrazolidinedione **12**. Oxidation of **12** with KHSO₅ afforded **13** in 90% yield. Final cleavage of **13** with Na/Hg furnished the desired product **14** in 74% yield. When using Et₂O as the precipitating solvent, the polar products would not be easily separated from the polymer upon cleavage. With the use of iPrOH this is no longer a concern. The synthesis of **14** is depicted in Scheme 2.



Scheme 2.

In a typical experimental procedure for the preparation of **12**, to a stirred solution of **11** (200 mg, 0.037 mmol) and PyBOP (117 mg, 0.23 mmol) in anhydrous DMF (6.0 mL) at rt under Ar was added methylhydrazine (14 μ L, 0.26 mmol) followed by EtN(iPr)₂ (120 μ L, 0.69 mmol). The resulting mixture was stirred at rt for 43h, and then evaporated to dryness, redissolved in MeOH (5.0 mL), and triturated with iPrOH (200 mL). The resulting white solid was filtered, washed with iPrOH (3 X 30 mL) and Et₂O (2 x 30 mL), and dried *in vacuo* to give **12** (196 mg, 98%). Also in a typical experimental procedure for the preparation of **14**, to a mixture of 5% Na/Hg (644 mg) and Na₂HPO₄ (40 mg, 0.28 mmol) at rt under Ar was added a solution of **13** (151 mg, 0.028 mmol) in anhydrous DMF (4 mL) followed by absolute MeOH (0.5 mL). The resulting mixture was stirred at rt for 18 h, and then Amberlite® IR-120(plus) (2.0 mL) was added followed by MeOH (2.0 mL). Stirring was continued at rt for an additional 2 h, then the mixture was filtered, washed with MeOH and the combined filtrate and washings were evaporated to dryness, redissolved in MeOH (4.0 mL), and triturated with iPrOH (180 mL).

The resulting white solid was washed with iPrOH and filtered. The combined filtrate and washings were evaporated to dryness, redissolved in EtOAc, and then flash chromatographed (hexanes/EtOAc, 1:1) to afford **14** (5.5 mg, 74%).

In summary, iPrOH was found to be a valuable solvent for the precipitation of PEG polymer, even in the presence of polar reagents and/or byproducts. The polymer-bound products could be readily isolated and inclusion complexes minimized by precipitation with iPrOH prior to release from the polymer. Moreover, polar compounds released from the polymer can easily be obtained by precipitation of the polymer with iPrOH and isolated from the iPrOH washings. This new purification system allows for the synthesis and isolation of polymer-bound 3,5-pyrazolidinedione from polar reagents and byproducts and the isolation of the cleaved 3,5-pyrazolidinedione from the polymer in good yields.

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References and Notes

1. Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489.
2. Han, H.; Wolfe, M. M.; Brenner, S.; Janda, K. D. *Proc. Natl. Acad. Sci.* **1995**, *92*, 6419.
3. a) The Organic Chemistry of Drug Synthesis; Lednicer, D.; Mitschnier, L. A.; Eds.; John Wiley and Sons, New York, Vol. 1, 1977, p 234. b) Buchanan, M. R. *Thromb. Res.* **1983**, Issue Suppl. IV, 89. c) Margulies, E. H.; White, A. M. *Pharmacol. Biochem. Prop. Drug. Subst.* **1979**, *2*, 255. d) Heming, A. E.; Kuzell, W. C. *Antibiot. Chemother.* **1953**, *3*, 634. e) Pfister, R.; Hafliger, F. *Helv. Chim. Acta.* **1961**, *44*, 232. f) Sherry, S. et. al. *N. Eng. J. Med.* **1978**, *298*, 289; **1980**, *302*, 250. g) El-Rayyes, N. R.; Al-Awadi, N. A. *Synthesis* **1985**, 1028.
4. Arriau, J.; Deschamps, J.; Teyseyre, J.; Maquestiau, A.; van Haverbeke, Y. *Tetrahedron* **1974**, *30*, 1225.
5. El-Rayyes, N. R.; Al-Awadi, N. A. *Synthesis* **1985**, 1028.
6. Zhao, X.; Janda, K. D. *Tetrahedron Lett.* **1997**, *38*, 5437.
7. Zhao, X.; Janda, K. D. *Bioorg. Med. Chem. Lett.* in press.
8. For recent review on polymer supported reactions see: (a) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. *Tetrahedron* **1997**, *53*, 5643. (b) Chaiken, I. M. and Janda, K. D. *Molecular Diversity and Combinatorial Chemistry, Libraries and Drug Design*; ACS: Washington, D. C., 1996; Conference Proceedings Series III.