Library-Based Lead Compound Discovery: Antioxidants by an Analogous Synthesis\Deconvolutive Assay Strategy

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Summary: A two-part demonstration project is reported which utilized analogous synthesis to reliably deliver a 27-analogue library which was then evaluated for antioxidative efficiency in a resin-free (ferric thiocyanate assay) deconvolutive assay.

Polymer-supported synthesis[1] has emerged as an important new synthetic strategy[2] in organic chemistry,[3] and the literature associated with polymer-supported tactics is extensive.[4] Further development of intriguing polymer-supported chemistry has been fostered by recent interest in the preparation of libraries of molecularly diverse compounds for deployment in various screening protocols. For the most part,[5] this screening of compound libraries has had a peptide focus wherein the diversity is constrained to amide and protecting group chemistries.[6] In this paper, we report the discovery of water soluble antioxidants by a strategy which couples analogous organic synthesis[7] and resin-free deconvolutive assay.

Figure 1 provides an overview of our two-part plan. Part one, analogue library synthesis, was envisioned to proceed from commercial Merrifield's peptide resin (® = chloromethylated styrene/2% divinyl benzene copolymer; ≈1 mequiv of Cl/g) to resin library **II** by two analogous synthetic steps consisting of esterification and aldol condensation. Subsequent reduction of resin library **II** would liberate the target 1,3-propanediol library (**III**) from the polymeric matrix. Part two embraces a resin-free deconvolution assay strategy (i.e., a strategy wherein the lead compound in a library is "discovered" by successive analysis of the analogue library and its predecessor sublibraries) to evaluate the antioxidative efficiency of analogues **III**.[8]

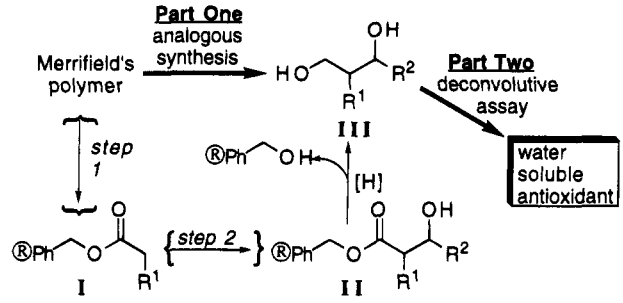
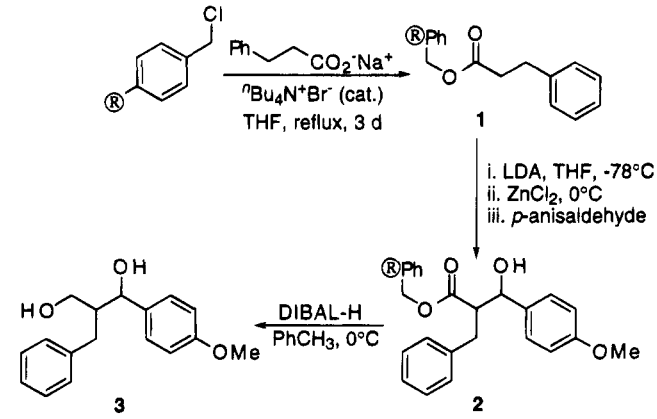


Figure 1.

Scheme 1



The chemistry targeted for the synthesis of library **III** was first explored as a solid-phase serial synthesis to establish the validity of each synthetic step (Scheme 1). For step 1, THF-swollen Merrifield resin (®$PhCH_2Cl$) was treated with sodium hydrocinnamate at reflux to effect carboxylate *O*-alkylation.[9] Following filtration and solvent wash, the resulting resin (**1**) was again swollen in THF, cooled to −78 °C, and treated with commercial lithium diisopropylamide (Aldrich; 2.0 M in heptane/THF/ethylbenzene, 90 min). A THF/anhydrous zinc chloride solution was added (30 min, 0 °C; the presumed

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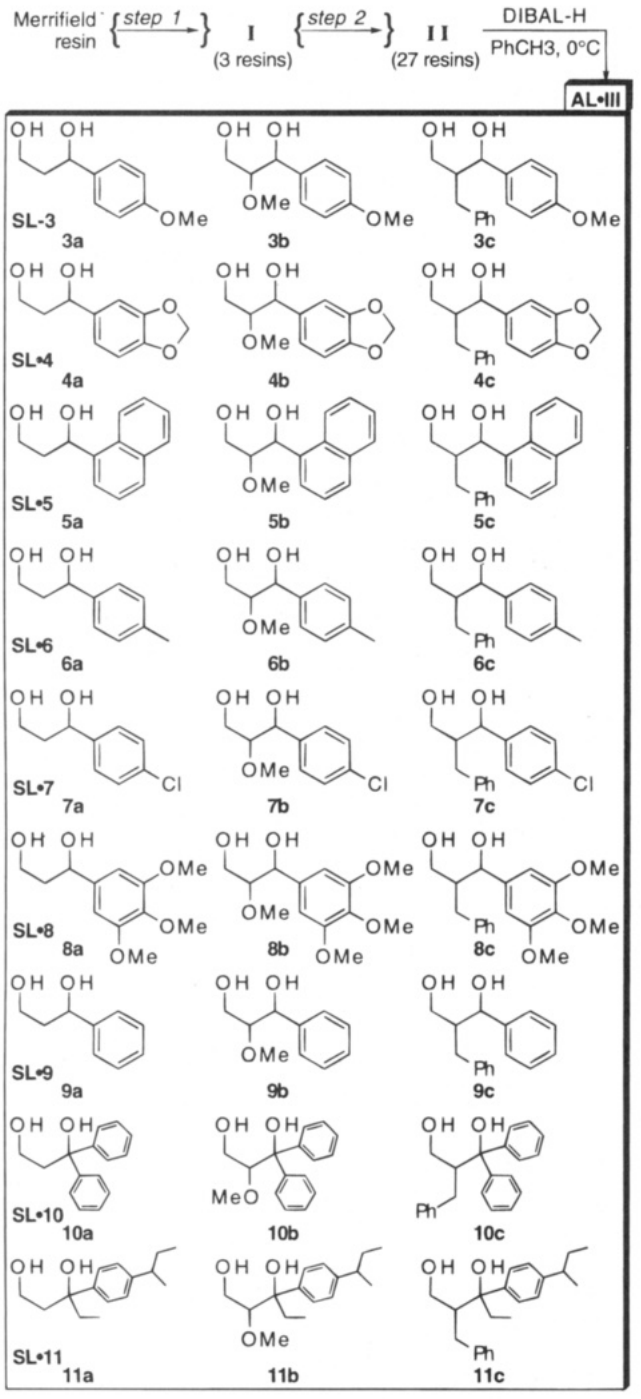
Scheme 2. Analogous Synthesis of Antioxidant Library III



zinc enolate minimizes retro-aldolization) followed by *p*-anisaldehyde (30 min, 0 °C). Addition of saturated aqueous ammonium chloride followed by filtration and solvent wash gave polymeric aldol product **2**. Since lithium aluminum hydride reduction of **2** resulted in significant retro-aldolization, diisobutylaluminum hydride (DIBAL-H) was selected for this ester reduction and diol **3** was obtained in a 7:5 ratio of *threo* to *erythro* products in 26% overall isolated yield from Merrifield resin. It is noteworthy that polymer-supported reactions to give **1** and **2** can be monitored by KBr pellet FT-IR analysis of the polymer.[10]

Scheme 2 details our extension of the above serial synthesis to the preparation of a library of 27 propanediol analogues. Analogous step 1 involved the split-vessel *O*-alkylation of sodium acetate, methoxyacetate, and

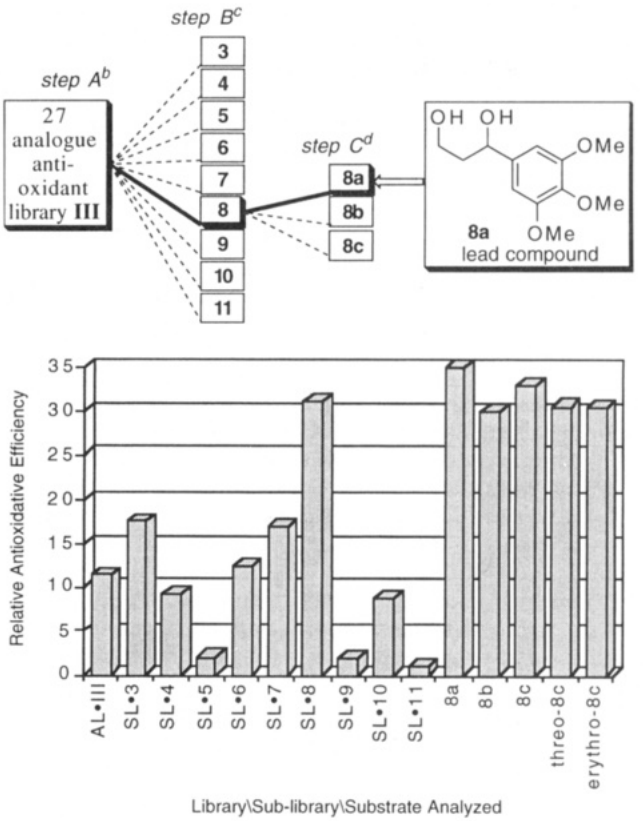


Figure 2. Deconvolutive assay to determine antioxidative efficiency.[a] (a) Key: efficiency expressed as a ratio to sublibrary **11** (**SL•11**) where maximum oxidation occurred; (b) step A, 1350 μg of library **AL•III**/mL; (c) step B, 150 μg of each sublibrary (**SL•3**−11)/mL; (d) step C, 50 μg of pure compound (**8a**−**c**)/mL.

hydrocinnamate with the chloromethyl moiety of Merrifield resin. The resulting resins were combined and mixed to give solid-phase pool **I** (−R^1 = −H\−OMe\−CH_2Ph) which was then equally partitioned into nine flasks for analogous step 2. Lithium diisopropylamide effected enolate formation and was directly followed by metal exchange ($ZnCl_2$) and aldol condensation with a series of seven aryl aldehydes (sublibraries **SL•3-9**) and two aryl ketones (sublibraries **SL•10** and **SL•11**). FTIR analysis was employed to establish that each sublibrary condensation had been achieved.

At this juncture, a portion of each three-product sublibrary was mixed to give solid-phase pool **II** which was subjected to diisobutylaluminum hydride reduction to liberate the propanediol derivatives. The resulting mixture was subjected to preparative thin-layer chromatography (TLC; hexane:EtOAc::50:50 eluent) and a broad band (i.e., everything excluding base line and solvent front) was eluted to give the targeted 27-analogue library **AL•III**. In a series of separate operations, a second portion of each three-product sublibrary was submitted to this reduction/preparative TLC procedure (appropriate hexane:EtOAc eluent selected to give a diol band with $R_f \approx 0.2-0.6$) and afforded the nine three-compound sublibraries (**SL•3**−**11**). All nine sublibraries were analyzed by low-resolution GCMS to verify that each targeted propanediol derivative was present in the relevant sublibrary and, by inference, in whole library **AL•III**.

(10) (a) Functional group changes were monitored by FT-IR (KBr pressed windows of ground polystyrene beads) as follows: **1** (C=O at 1739 cm^{-1}) → **2** (OH at 3575, 3482 cm^{-1} and C=O at 1736 cm^{-1}). (b) Frechet, J. M.; Schuerch, C. *J. Am. Chem. Soc.* **1971**, *93*, 492−6.

The ferric thiocyanate method,[11] a colorimetric assay that measures the amount of linoleic acid hydroperoxide in an emulsion system, was used in a deconvolutive assay of our antioxidant analogue library and sublibraries. Briefly, after an analogue pool (library or sublibrary) was incubated for a period of 3 days, an aliquot of the sample solution was mixed with ammonium thiocyanate plus iron(II) chloride and the 490 nm absorbance of the resulting red color was determined as a measure of the degree of oxidation present. We have developed this assay in a 96-well format, yielding a rapid, reliable method for screening which maximizes sensitivity versus antioxidant sample size.

A schematic view of this deconvolution approach is presented in Figure 2. Step A, ferric thiocyanate assay of antioxidant library **AL·III**, established that this pool of 27 compounds afforded meaningful antioxidative efficiency (inhibition of color development) and thus warranted ferric thiocyanate assay of each sublibrary (step B). As illustrated in the bar graph (Figure 2), the nine sublibraries **SL·3−11** afforded disparate antioxidative efficiency with sublibrary **SL·8** (compounds **8a**,[12] **8b**, and **8c**) producing the least color development in quantitative studies. The three analogues of this lead sublibrary were then prepared serially from the acetate, methoxyacetate, and hydrocinnamate resins and submitted for individual analysis (step C; **8b** and **8c** were ≈1:1 mixtures of diastereomers). The ferric thiocyanate assay results in step C indicate that all three trimethoxy analogues have comparable antioxidative efficiency.[13] Furthermore, the *threo* and *erythro* isomers of **8c** respond similarly in this assay.

In conclusion, these results demonstrate that analogous synthesis coupled with a resin-free deconvolution assay can lead to the discovery of compounds with targeted activity. This demonstration project utilized analogous synthesis to reliably deliver 27 targeted analogues which were then evaluated for antioxidative efficiency in a resin-free ferric thiocyanate assay. Analogous synthesis studies coupled with resin-bound assays are currently in progress and will be reported shortly.

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Supplementary Material Available: Experimental details for the preparation of **3c** and **8a−c**, experimental details for the preparation of library **AL•III** and sublibraries **SL•3−SL•11**, low-resolution mass spectrometry data for **3a-11c**, and ^{13}C-NMR spectra for **3c** (both diastereomers), **8a**, **8b**, and **8c** (both diastereomers) (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(13) Water solubility data: **8a** and **8b** are infinitely miscible with water, and **8c**'s water solubility is ~3 mg/mL.