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A convenient approach for solution-phase synthesis of water-soluble galactoside libraries

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Abstract—A convenient approach for the solution-phase synthesis of water-soluble galactoside libraries through tributylphosphine promoted reaction of azido compounds with carboxylic acids is described.

In the past decade, combinatorial chemistry has emerged as a powerful tool for drug discovery research.[1] Its ability to generate large numbers of structurally diverse compounds (a library) within a limited period of time greatly enhances the efficiency of lead-compound exploration. While early combinatorial chemistry has primarily focused on solid-phase synthesis, parallel library synthesis performed in solution phase has become increasingly popular. Facing every solution phase library synthesis is the need of efficient purification methods. Consequently, a handful of innovative purification approaches have been unveiled. These include resin capture,[2] acid/base wash,[3] C18-silica extraction,[4] and most notably, the fluorous phase synthesis.[5] We herein report a convenient approach for the solution-phase parallel synthesis of water-soluble galactoside derivatives. Such an approach was designed by carefully choosing conditions so that all reagents and by-products are either volatile or soluble in organic solvent. Hence, the water-soluble products could be obtained conveniently after removal of all by-products and excess reagents by partitioning in water/organic solvent and evaporation.

We demonstrate this approach by using an amide bond formation reaction, which generates a library of galactoside derivatives (**5**, **6**, **7** in Scheme 1). Libraries of this type can be used to explore the inhibition of the receptor-binding process of bacterial toxins produced by enterotoxigenic *E. coli* and by *V. cholerae*.[6]

Among many amide bond formation reactions,[7] we found that trialkylphosphine promoted reaction of azido compounds with carboxylic acids[8] involves only organic soluble or volatile reagents and by-products. No intermediate purification step is needed. The water-soluble products are generated only in the final deprotection step with facile purification.

The analysis of reaction process is demonstrated in Scheme 2. The first amide bond formation step was carried out by using equal amounts of a carboxylic acid and tributylphosphine in CH_2Cl_2, both in slight excess to the azide (**1**) to ensure complete consumption of the azide. Along with the desired intermediate (**8**), the reaction also formed by-products: galactosylphosphazene (**9**), tributylphosphine oxide, as well as the unreacted carboxylic acid and tributylphosphine. After the removal of CH_2Cl_2, the mixture was directly subjected to deprotection using NaOMe/HOMe, followed by neutralization with Dowex-H^+ resin. Besides the water-soluble product (i.e. library compound **5**), methyl acetate and 1-amino-1-deoxy-galactose (**10**) were generated in this step. During the Dowex-H^+ resin treatment, **10** and Na^+ were bound to the resin and filtered off. Subsequently, methanol and the by-product methyl acetate were evaporated. At this stage, all the remaining contaminants (tributylphosphine oxide, tributylphosphine and the acid) are soluble in organic solvent and were removed by partitioning in CH_2Cl_2/H_2O. The water-soluble product was obtained after lyophilization.

Initially, the complete library synthesis (using **1**, **2**, **3** with 24 acids: 72 members) was performed at room temperature without pre-drying the starting materials. A typical procedure is described with compound **1**.[9]

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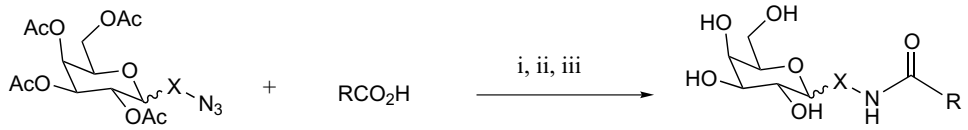
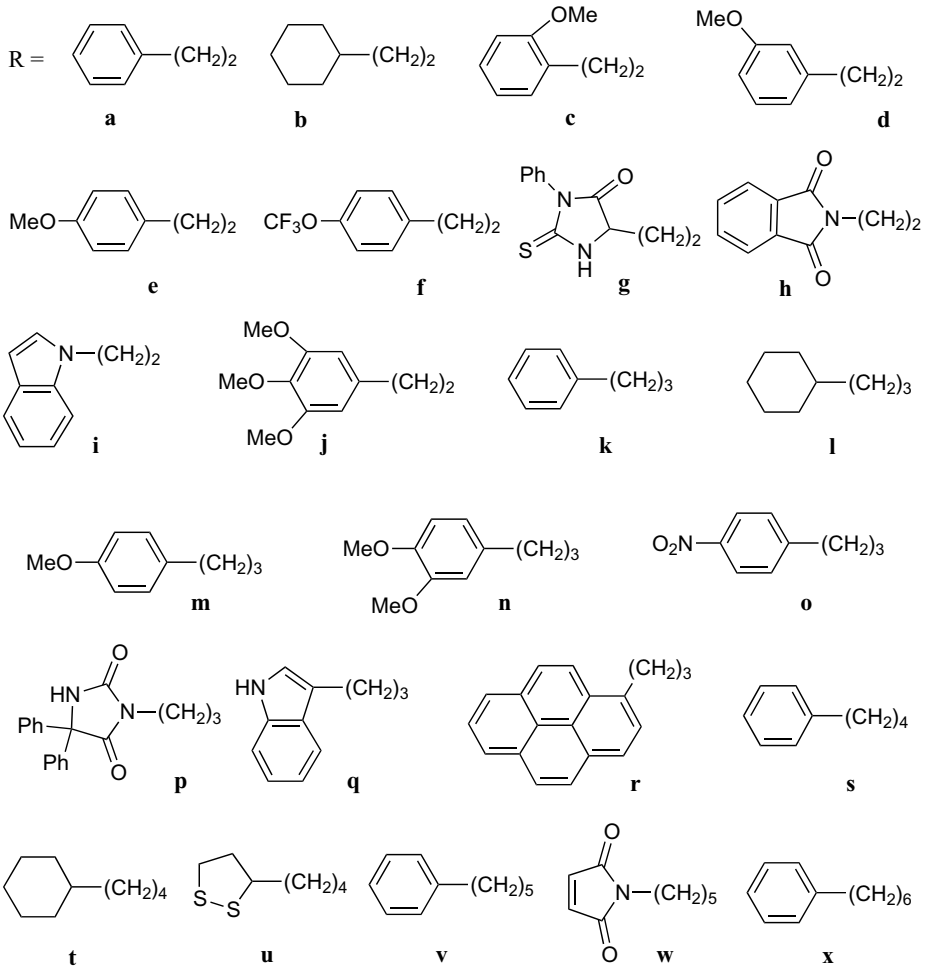


1, 2, 3 **4** **5, 6, 7**

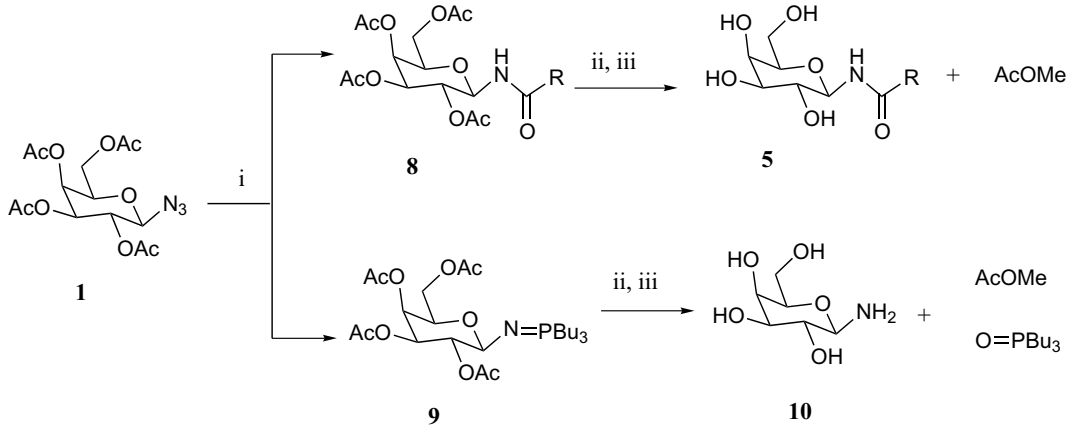
i). n-Bu_3P, CH_2Cl_2, rt; ii). NaOMe/HOMe, rt; iii). Dowex-H^+, HOMe, rt

1, 5: X = none, β **2, 6**: X = OCH_2CH_2, α **3, 7**: X = OCH_2CH_2, β



Scheme 1.

Overall yields ranged from 8.2 to 44% with high purity of the final products for screening.[6b] All reactions were monitored by TLC. The purities of the final compounds were determined by HPLC and TLC. Most final products had purities between 70 and 95% based on HPLC monitored at 220 nm, while TLC indicated purities of >95% (single spot with ammonium molybdate staining). Unreacted carboxylic acids were the major contaminants as identified using HPLC analysis by co-injection with starting acids. All products gave the correct mass spectra as determined by MALDI or ESI mass spectroscopy.[10] In the initial synthesis, the overall yields were quite disappointing, especially for library compounds starting with the less reactive azide **1**. Efforts were made to improve the overall yields by varying reaction conditions, including temperatures, solvents, and reagents such as using triethylphosphine as promoter. However, no dramatic improvement in yield was observed. We noticed that pre-drying of starting materials by co-evaporation with toluene helped to improve the isolated yields moderately to ~15–55% in a later round of library synthesis using azides **1**–**3** and acids **4k**, **4n** and **4o**. These yields are in good agreement with the general yields obtained for reactions carried out at room temperature.[8] Inazu et al. performed a thorough investigation of reaction conditions and mechanisms for this type of amide bond transformation.[8b] It was suggested that carrying out the reaction at very low tem-



Scheme 2. (i) RCO_2H, n-Bu_3P, CH_2Cl_2, rt; (ii) NaOMe/HOMe, rt; (iii) Dowex-H^+, HOMe, rt.

perature (−78°C) can facilitate the formation of desired amide products, while reaction at higher temperature (such as room temperature) can lead to the non-productive glycosylphosphazene derivatives (**9** in Scheme 2). From a library synthesis point of view, we did not attempt to run the library synthesis at −78°C in the absence of specialized equipment. In our library synthesis at room temperature, we did observe the formation of significant amounts of galactosylphosphazene derivatives. Despite the presence of large amounts of phosphazene by-products, our purification procedure can efficiently remove these impurities. At a small expense of the yield, the use of excess Dowex-H^+ resin ensures the breakdown of phosphazenes and the subsequent trapping of generated amines.[11] For efficient removal of the remaining neutral organic by-products, we also found that in addition to CH_2Cl_2, ethyl acetate can also be used to perform extraction from water.

In summary, we have described a convenient approach for the solution-phase synthesis of water-soluble galactoside libraries. By carefully choosing a reaction, involving either organic-soluble or volatile reagents and by-products, we have efficiently obtained water-soluble pure galactoside derivatives through simple organic solvent extraction, evaporation and lyophilization despite of the low yielding nature of the reaction. The convenience and efficiency might make such an approach a useful alternative to the solution-phase library synthesis of various water-soluble organic compounds.

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9. Typical procedure: To each acid (0.074 mmol) in a 1 dram glass vial was added **1** (25 mg, 0.067 mmol) in 1.5 ml of anhydrous CH_2Cl_2. After shaking for 5 min, n-Bu_3P (0.074 mmol) in 0.5 ml of CH_2Cl_2 was introduced via a syringe through the cap. The resulting mixture was shaken overnight at room temperature. All vials were then placed in a desiccator that was connected to vacuum to remove the solvent. To each residue in the same vial was added 1.5 ml of methanol, followed by NaOMe/

HOMe (6.7 mmol). After 1 h, reaction mixture was treated with Dowex-H^+ resin (400 mg). The resin was filtered off and the filtrate was collected in a 20 ml vial. Methanol was evaporated the same way as for CH_2Cl_2. The residue was partitioned in 10 ml of H_2O and 5 ml of CH_2Cl_2 with stirring for 5 min. The CH_2Cl_2 layer was removed, and the extraction was repeated once. Water solution was subjected to lyophilization to give the final product.

10. After library synthesis, all compounds were checked by mass spectroscopy using either MALDI or electrospray ionization. All library samples gave the corresponding $(M+H)^+$ and/or $(M+Na)^+$ peaks. The purity of each sample was determined by reverse phase HPLC using a C18 column with gradients starting with 10% (v/v) CH_3CN in 0.1% aqueous TFA and reaching 60% CH_3CN in 0.1% aqueous TFA in 20 min. For the nine compounds in the second round of library synthesis, the HPLC gradient was from 0% CH_3CN in 0.1% aqueous TFA to 50% CH_3CN in 0.1% aqueous TFA in 30 min. HPLC peaks were monitored by UV detector at 220 nm and at another wavelength where the starting acids have significant absorbance.
11. Excessive amounts of Dowex resin can lower the isolated yields of final products as the resin also traps product non-specifically. For example, treatment of 30 mg of a phosphazene-free product in 5 ml MeOH with 1 ml of dry-volume Dowex resin can cause $\sim$30% loss of weight after filtration and evaporation of solvent.