Preparation of the Neolignan Natural Product Grossamide by a Continuous-Flow Process

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Received 12 December 2005

Abstract: This article describes the first enantioselective total synthesis of 2-aryl-2,3-dihydro-3-benzofurancarboxyamide neolignan, grossamide (**1**) using a fully automated and scalable flow reactor.

Key words: polymer-supported reagents, enzymes, flow synthesis, natural products

Many plants respond to pathogenic attack such as fungal infection by the rapid biosynthesis and accumulation of low molecular weight antimicrobial compounds. A significant proportion of these compounds are oligomeric hydroxycinnamic amide derived metabolites. Lignanamides, a structurally diverse class of compounds possess both cyclic[1] and acyclic[2] structures many of which have been shown to regulate biological functions in both plants and microorganisms making them interesting lead discovery candidates for both pharmaceutical and agrochemical investigation.[3] As part of our present research into this class of compounds we required a rapid and flexible method of preparing such compounds at various scales as an on-demand process. We also wished to avoid the need for extensive manual purification of the intermediates and final products within an expanded library format. Our vision was that this could be achieved through an integrated sequential flow synthetic pathway employing immobilized reagents to mediate each individual transformation in the sequence.[4]

Herein, we wish to report on the design and validation of an automated flow reactor capable of synthesizing gram quantities of compound that can also be used for the preparation of small focused libraries. A schematic of the flow reactor indicating its major components is shown in Figure 2. The system was designed in a modular fashion in order to permit the rapid alteration of its configuration and allow evolution of its functions.

For our simple proof of concept work we chose to prepare the neolignan grossamide (**1**, Figure 1) an interesting member of the lignanamide family. Grossamide (**1**)[1b,1e,2f,2g,3b,5] and its *N*-feruloyltyramine building block **2**[2,6] have been isolated as major constituents of various fruit and seed sources (Figure 1). Mechanistically, grossamide (**1**) can be prepared from an amide coupling of tyramine and ferulic acid followed by oxidative dimerization and intramolecular cyclization. Such a proposed synthetic cascade would also be consistent with the biosynthesis as extrapolated from related systems and the characterization of isolated intermediates and related by-products.[1e,2b,6] It was our aim to demonstrate such a sequence could be conducted in a flow mode[7] using columns of immobilized reagents.

The functionalization of a prepacked column of polymer-supported HOBt (PS-HOBt) as the activated ester was achieved by eluting the column (Column 1, Figure 2) with a solution of ferulic acid, PyBrOP and DIPEA (two-fold excess, premixed before entering the column) in DMF. The column was then washed to remove any unreacted coupling materials.[8] At this stage the column (Column 1) could be switched to a second channel and a second column (Column 2) brought into line to be loaded using the same procedure. Meanwhile a solution of the amine coupling partner, tyramine in THF was directed through the first column (Column 1) reacting as it flowed through to

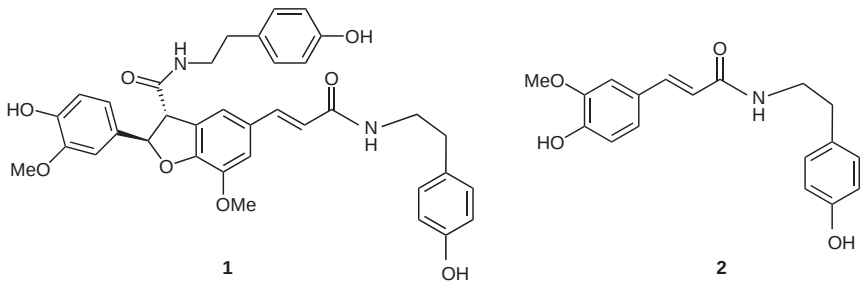


Figure 1 Grossamide (**1**) and *N*-feruloyltyramine (**2**)

SYNLETT 2006, No. 3, pp 0427–0430
Advanced online publication: 06.02.2006
DOI: 10.1055/s-2006-926244; Art ID: D37305ST

yield the amide adduct **2**. This process was finally monitored using very simple inline UV/Vis detection. However, it was initially rapidly optimized by routing the flow to the LCMS sampling stage. This indicated that we could cleanly elute upwards of 75% of the theoretical maximum loading of the PS-HOBt activated ester before any contamination of the output stream with unreacted amine was detected.[9] Alternatively, the conversion could be increased to almost 90% (5–8% contamination of amine present) and the reaction stream directed through an inline scavenging cartridge containing a sulfonic acid resin (Column 3). This was envizaged as a desirable extension of the synthesis process as an exemplification, which would be extremely valuable when possessing more precious materials. As a proof of concept capturing the unreacted amine in this way offers an increased guarantee of the amide's purity but in addition it facilitates a very easy way of recovering the amine through subsequent release from the trapping resin. Obviously this material could then be easily recycled. Indeed this principle has been achieved on our simple tyramine substrate by eluting the scavenging column with an excess of triethylamine (TEA) in THF. Although this function is not included in the reactor schematic for simplicity (Figure 2), the process was easily performed by switching the input flow (via an multi-position valve) to a pumped TEA solution (at the same time bringing a replacement scavenging cartridge inline) and diverting the output to an alternative collection vessel in an entirely automated fashion.

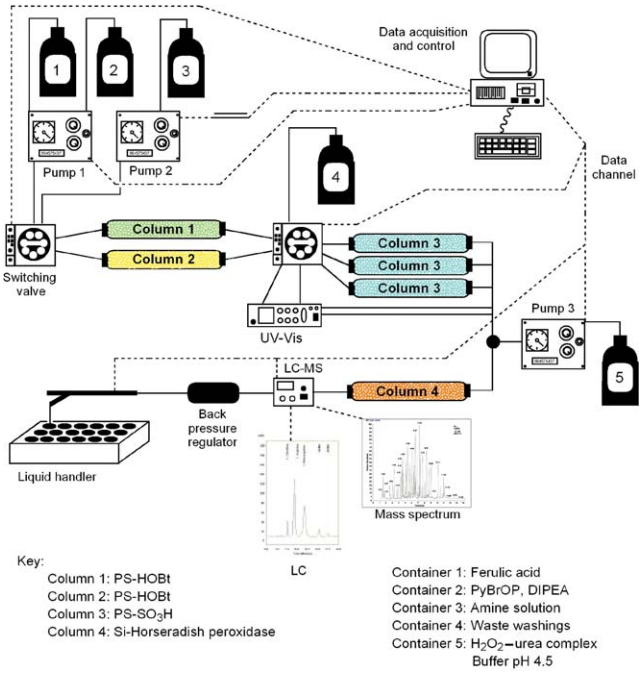
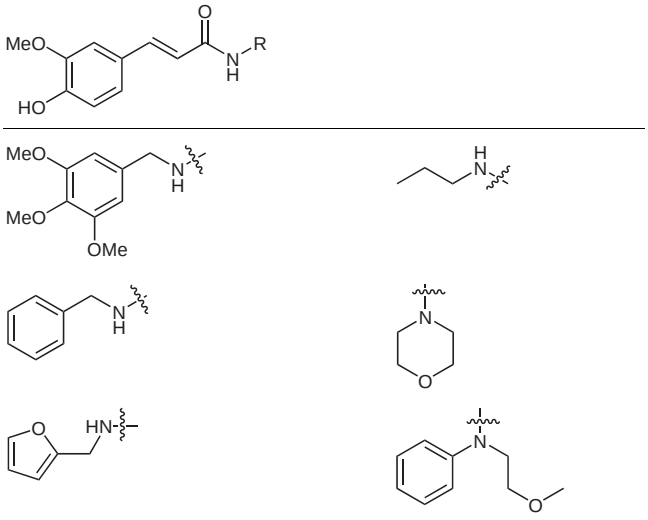


Figure 2 Schematic representation of the flow reactor

Sequencing the timings to allow consumption of the immobilized active ester (Column 1) to coincide with the activation of the second PS-HOBt packed column (Column 2) enables a seamless and fully automated exchange of the columns to give uninterrupted processing. The now depleted PS-HOBt column (Column 1) can again be loaded with the desired acid, as before, ready for reincorporation into the reacting flow stream.[10]

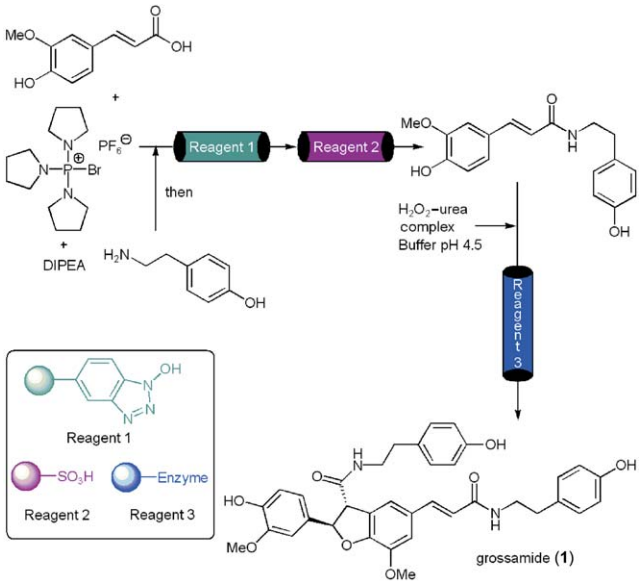
As well as being able to continually produce the same amide material we have also demonstrated that we can sequentially react various amine components in flow generating different amide products. This can even be performed through the same activated ester column without any cross contamination of the products using a short washing sequence of an aliquot of clean solvent between each alternative substrate. Table 1 shows a short summary of the materials prepared. Indeed placing a blank solvent marker between each sample provides a very simple triggering signal change for automated fraction collection of each product by a liquid handler using a simple technique such as UV/Vis detection. In addition, whereas each of the columns (columns 1 and 2) has been loaded with the same acid, in principle, each could contain a separate substrate giving multiple variations in the processing of new amides.

Table 1 Substrates Used in Amide Coupling



For the synthesis of grossamide (**1**) the flow stream of precursor **2** following its passage through the sulfonic acid scavenging cartridge (Column 3) was diluted (3:1) with a second input solution containing hydrogen peroxide urea complex and sodium dihydrogen phosphate buffer (pH 4.5) in acetone–water (1:4). The combined flow was then passed into a fourth column (Column 4) packed with an immobilized peroxidase enzyme. We have previously demonstrated the effectiveness of using immobilized enzymes within multistep sequences, especially for classically difficult synthetic transformations.[11] Again, this was found to be a very efficient strategy for the synthesis of grossamide (**1**) using in this latter case an immobilized horseradish peroxidase (type II) on silica. Automatic sampling (LCMS analysis) directly from the product reaction stream gave an effective way of assessing the reaction progress. This allowed direct feedback

and tailoring of the flow rates, dilution ratios and reagent concentrations. Ultimately, this enabled retroengineering and calibration back through the previous transformations in order to sequence the entire operation for optimized production of the desired compound **1**.[1b,1e,2f,2g,3b,5] The final synthesis can be depicted as shown in Scheme 1 in a simple processing diagram.



Scheme 1 Simple flow synthesis of grossamide (**1**)

In summary we have constructed a fully automated continuous flow reactor system using a simple pumping arrangement with immobilized reagents packed in columns to effect the efficient synthesis of the neolignan natural product grossamide (**1**). We believe, however, that the concepts we have developed here will have a much wider impact on the multistep assembly of a much wider range of chemical substances.

Acknowledgment

We gratefully acknowledge the financial support from the RS Wolfson Fellowship (to S.V.L. and I.R.B.), Natural Sciences and Engineering Research Council of Canada for a Postdoctoral Fellowship (to G.K.T.), GlaxoSmithKline (to C.M.G.J.) and the BP endowment and the Novartis Research Fellowship (to S.V.L.).

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(8) With DMF followed by THF.

(9) The exact amount was highly dependant on flow rates, scale and specific dimensions of the column. Optimum conditions could be rapidly determined in an automated screening fashion.

(10) The columns could be efficiently recycled up to five times before any noticeable decrease in reactivity was observed.

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