Carbohydrate Research 418 (2015) 98–103

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

A new method testing the orthogonality of different protecting groups

Károly Ágoston [a,b,*], Ágnes Ágoston [c], Colin R. Dorgan [b], Péter Fügedi [a]

[a] Institute of Organic Chemistry, RCNS-HAS, Magyar Tudósok Körútja 2, 1117 Budapest, Hungary
[b] Carbosynth LTD, 8&9 Old Station Business Park, RG20 6NE Compton, UK
[c] Lab-Comp LTD, Rákos Tér 23/A, 1142 Budapest, Hungary

ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form 16 October 2015
Accepted 16 October 2015
Available online 23 October 2015

Keywords:
Protecting groups
Orthogonal protection
Method development

ABSTRACT

A new test was elaborated to identify a new set of orthogonal protecting groups. With the developed method eight different protecting groups were tested under various deprotection conditions and the complex reaction mixtures were analysed by HPLC. The developed method allows for quick identification of orthogonality using simple model structures.

1. Introduction

The synthesis of complex natural products generally requires the extensive use of protecting groups. Although very elegant, there are few examples of natural product total-synthesis which avoid the use of any protecting groups at all.[1] These examples are exceptions to the trends in synthetic chemistry. The number of protecting groups used during a synthesis grows significantly with the increasing complexity of the targeted compound. The concept of orthogonal sets of protecting groups has been established for more than 30 years[2] for the protection of amino groups in peptide synthesis. The principle has been generalized for practically all kinds of protecting groups.[3] The concept of orthogonal protection is particularly useful for the synthesis of complex branched oligosaccharides. Already several orthogonal sets have been reported and applied for the protection of the hydroxyl groups within oligosaccharide synthesis.[4] Most of these sets consist of 2–4 individual groups. We believe there is still a need for new sets of orthogonal protecting groups especially with sets of more than four members. To our knowledge there is only one example in the literature to use five orthogonal protecting groups during a synthesis.[5] The increasing number of newly developed protecting groups makes it possible to identify similar orthogonal sets with more than four orthogonal protecting groups. In order to develop new sets of orthogonal protecting groups, time consuming preparative work is necessary until a fully protected derivative is made. Our intention was to shorten this procedure with a fast and simple test method.

2. Results and discussion

The aim of this study was to develop a quick and simple method to test the orthogonality of selected protecting groups. The basis of our method can be seen in Fig. 1. Compound **A** was selected as a starting material having one free hydroxyl function. (Protected monosaccharide derivative was selected for the study which is available in three steps from raw materials.) R and R′ were persistent protecting groups and at least one of the groups is aromatic providing good UV absorbance for detection by HPLC. The free hydroxyl function of compound **A** was protected with different temporary protecting groups affording derivatives such as $\mathbf{B_1}$ or $\mathbf{B_2}$. The applied temporary protecting groups were tested for their orthogonal behaviour. Known literature methods to remove those temporary groups were optimized on the clean $\mathbf{B_i}$ derivatives. An HPLC method was developed to separate all the $\mathbf{B_i}$ derivatives and **A** starting material. Then all $\mathbf{B_i}$ derivatives were mixed in equal molar concentrations resulting in a stock solution. The optimized conditions to remove temporary protecting groups were applied to samples of the stock solution and after work up procedures the crude reaction mixtures were analysed by HPLC methods.

Methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (**A**)[6] was selected as scaffold for derivatization (Scheme 1). Compound **A** is easily available from methyl α-D-glucopyranoside in three steps with literature methods[7] and protected with benzyl groups which are commonly used as persistent protecting groups. Furthermore these groups provide high UV absorbance for detection in HPLC analysis.

* Corresponding author. Carbosynth LTD, 8&9 Old Station Business Park, RG20 6NE Compton, UK. Tel.: +44 07840347437; fax: +44 01635579444.
E-mail address: karoly.agoston@carbosynth.com (K. Ágoston).

http://dx.doi.org/10.1016/j.carres.2015.10.007
0008-6215/

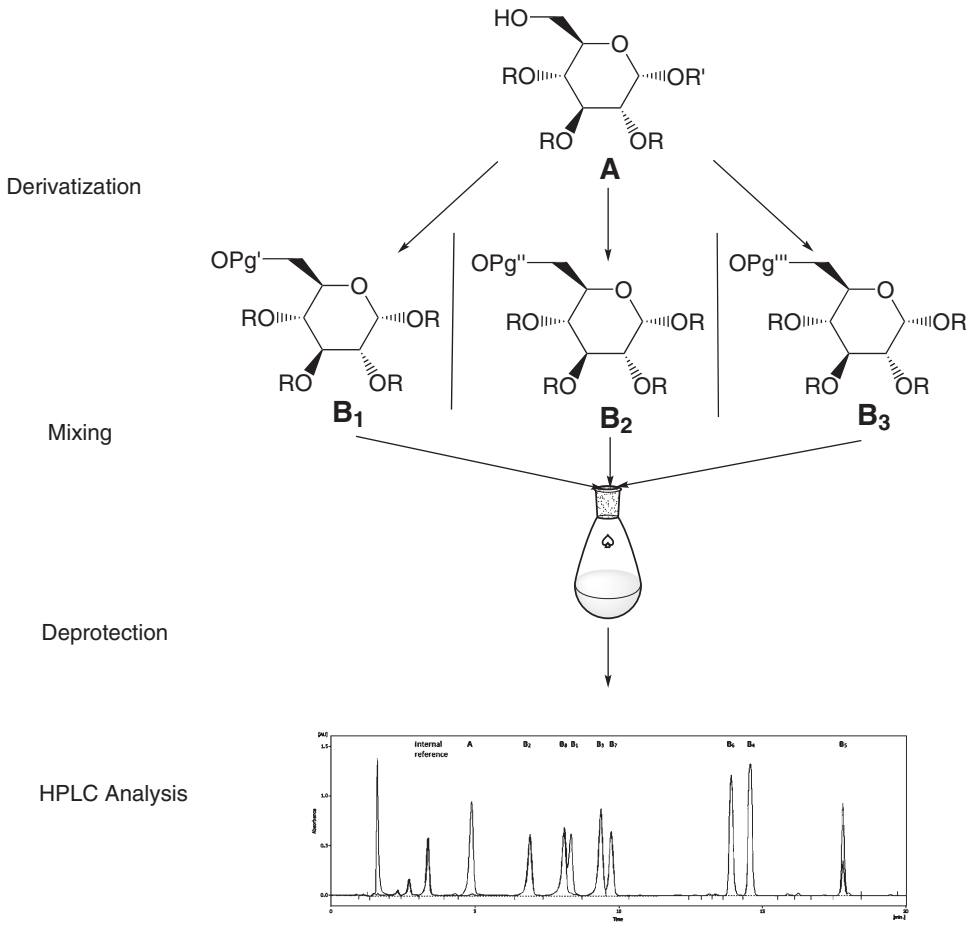

Fig. 1. The principle of the test.

The temporary protecting groups selected for the test were chloroacetyl, levulinoyl, (*o*-nitrophenyl)acetyl esters, Fmoc carbonate, *t*-butyldimethylsilyl, 1-naphthylmethyl, allyl and propargyl ethers (Scheme 1). Some of these groups have been known to be orthogonal (e.g. chloroacetyl, levulinoyl, Fmoc) to each other for many years,[3] while other groups were proven to be orthogonal recently.[8] All of $\mathbf{B_i}$ derivatives were prepared based on literature procedures. Known methods for the chemoselective cleavage of the protecting groups were optimized for $\mathbf{B_i}$ derivatives (see the details in the experimental part of the Supplementary material). All $\mathbf{B_i}$ derivatives were mixed in a defined concentration resulted in a stock solution. An HPLC method was developed to separate all derivatives including compound **A**. Calibration curves were recorded to obtain more precise calculation. Unfortunately, there was no baseline separation between 6-propargylated and 6-chloroacetylated derivative but this did not affect the calculation based on the calibration curves. All deprotection conditions were performed on the stock solution (see the details in the Experimental section), and the HPLC results collated into Table 1. The numbers in each field represent the percentage of the $\mathbf{B_i}$ present in the mixture after performing the deprotection procedure. The numbers were rounded to the nearest 5% value for easier interpretation (for the exact obtained numbers, see Supplementary information).

First the removal of the chloroacetate group was tested, and under the developed conditions all $\mathbf{B_1}$[9] was transformed into compound **A** (Table 1, entry 1, for HPLC chromatogram see Fig. 2). As an undesired effect we observed the partial decomposition (deprotection) of the silylated derivative ($\mathbf{B_5}$). Thiourea treatment alone on the pure $\mathbf{B_5}$ did not result in any cleavage of the silyl protecting group, but the HCl generated in the reaction between thiourea and the chloroacetylated derivative caused the partial cleavage of the silyl ether.

This was completely suppressed by the addition of pyridine into the reaction mixture (Table 1, entry 2). This undesired, but expected side effect showed the effectiveness of our test method as it is not only the added reagent which is able to cleave certain protecting groups. Other reactive species can form during a reaction, which might interact with other compounds causing side-reactions. These circumstances would not be observable when working with clean compounds separately, therefore our method provides a more comprehensive test of protecting group orthogonality.

The cleavage of the Lev ester group from compound $\mathbf{B_2}$ did not cause any degradation of other protecting groups and resulted in high recovery of the individual compounds (Table 1, entry 3, for HPLC chromatogram see Fig. 3). Under the conditions to cleave NPAc ester group ($\mathbf{B_3}$[8] → **A**) most of the protecting groups were not decomposed at all, but the two other esters (chloroacetate and levulinate) present in the mixture were damaged to some extent (Table 1, entry 4). The decomposition of these ester groups was not significant, but already visible on the HPLC chromatogram. Most probably the amine generated during the cleavage was the reactive agent causing these decompositions. Cleavage of Fmoc carbonate ($\mathbf{B_4}$ → **A**) with diluted, hindered base (DBU) and the cleavage of silyl ether ($\mathbf{B_5}$[10] → **A**) with diluted acid (camphorsulfonic acid) resulted in very clean reaction mixtures with no observed decomposition of other protecting groups (Table 1, entry 5 and 6, respectively).