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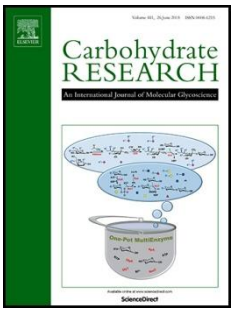

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A new method testing the orthogonality of different protecting groups

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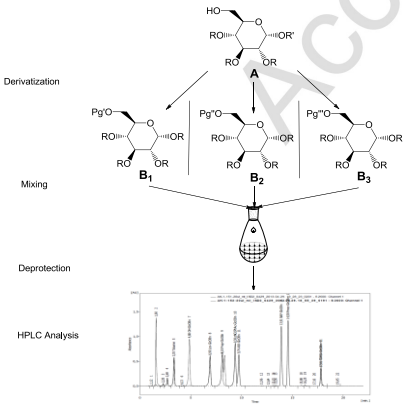
Highlights

- A method was developed to identify orthogonality of various protecting groups.
- The method is based on the chemo-selectivity of protecting groups.
- The crude reaction mixtures were analysed by HPLC.
- With the method a new deprotection protocol for the propagyl group is developed.

Graphical abstract



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.

Keywords
Protecting groups, Orthogonal protection, Method development

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 Figure 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. A 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.

The temporary protecting groups selected for the test were chloroacetyl, levulinoyl, (*o*-nitrophenyl)acetyl esters, Fmoc carbonate, *t*-butyldimethylsilyl, 1-naphtylmethyl, 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 info). All $\mathbf{B_i}$ derivatives were mixed in a defined concentration resulted in a stock solution. A 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 part), 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 Supporting Info).

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 Figure 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 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 Figure 3). Under the conditions to cleave NPAc ester group ($\mathbf{B_3}^8 \rightarrow \mathbf{A}$) 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 ($\mathbf{B_4} \rightarrow \mathbf{A}$) with diluted, hindered base (DBU) and the cleavage of silyl ether ($\mathbf{B_5}^{10} \rightarrow \mathbf{A}$) 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).

Cleavage of the naphtyl ether group under the oxidative conditions ($\mathbf{B_6} \rightarrow \mathbf{A}$) developed for the clean product did not result in complete cleavage of the NAP ether group when applied to the stock solution. (Table 1, entry 7) The amount of the reagent used for the reaction and the reaction time had to be increased to achieve full conversion. (Table 1, entry 8). Due to the hydrolytic reaction conditions the silyl ether group was decomposed completely even with the reduced amount of reagent.

Under the conditions to remove allyl ether protecting groups ($\mathbf{B_7}^{11} \rightarrow \mathbf{A}$) most of the other protecting groups were untouched (Table 1, entry 9). One of the exceptions was the silyl ether group which was completely cleaved due to the hydrolytic condition used to cleave the rearranged enol-ether. The other compound which completely disappeared from the reaction mixture was $\mathbf{B_8}$, the propargyl ether protected derivative. This observation could have been expected since transition metal based catalyst might interfere with triple bonds. According the accurate calculations on the amount of compound **A** not all $\mathbf{B_8}$ was transformed into **A**.

In the literature there are different conditions to remove propargyl ether,[12] but most of these are not compatible with ester protecting groups due to the strongly basic conditions. The condition we selected was based on the formation of a pyramidal complex of the triple bond with dicobalt-octacarbonyl.[12h] The formed complex can be hydrolyzed under acidic conditions revealing the free hydroxyl function ($\mathbf{B_8}^{12a} \rightarrow \mathbf{A}$). Unfortunately, strongly acidic conditions were needed to cleave the ether bond. This was modestly tolerated by other protecting groups (Table 1, entry 10). There were two groups with relative high stability under this condition, namely the Fmoc carbonate and the [1]NAP ether protected derivatives, but we can conclude that the conditions we used for the deprotection of the propargyl group is not highly compatible with other protecting groups.

3. Conclusion

A powerful method was developed for testing the stability of different protecting groups under various reaction conditions. HPLC methods were applied to analyze the crude

reaction mixtures. Eight different protecting groups were used for the study and all deprotection conditions developed for the individual protecting groups were tested. New sets of orthogonal protecting groups could be identified with the presented method. Based on the results a new set of orthogonal protecting groups could be used such as: chloroacetyl, levulinoyl, (*o*-nitrophenyl)acetyl esters, Fmoc carbonate, 1-naphtylmethyl and allyl ethers. This model system is ideal to test the orthogonality of selected protecting groups before starting any synthesis using an unknown combination of different protecting groups. The method is based only on the chemo-selectivity of the various protecting groups. The main limitation of this method is the use of a primary hydroxyl group for the test as primary hydroxyls generally have higher reactivity than the more common secondary. We still assume that the results generated on this system can be interpreted more generally by using different reaction conditions (for example performing stability test using different concentration of reagents and vary temperatures or reaction times). It is beyond the scope of this study to deal with migration of acyl groups and further side reactions which are structure dependent reactions, thus influenced by the orientation of the protected hydroxyl groups. The method, as our referee suggested, could be potentially used to test more challenging set, such as selective deprotection of different silyl ether groups. In addition to identifying a new set of orthogonal protecting groups the test could provide new deprotection protocols for certain protecting groups – as in our case turned out with the propagyl ether group. The investigation of propagyl ether group deprotection with Wilkinson's catalyst, as a new method, will be published separately.

4. Experimental

4.1. General. — Commercially available starting materials were used without further purification. Solvents were dried according to standard procedures. Melting points (uncorrected) were determined on a Griffin apparatus. Optical rotations were measured with a Jasco-Optical activity AA-10R polarimeter. NMR spectra were recorded on a Varian Gemini 2000 (200 MHz for ^{1}H and 50 MHz for ^{13}C) and on a Varian Unity-Inova (300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometer in $CDCl_3$ as solvent. All chemical shifts are quoted in ppm downfield from the characteristic signals (^{1}H: 0.00 ppm (TMS), ^{13}C: 77.00 ppm ($CDCl_3$)). Kieselgel 60 (E. Merck, Darmstadt, Germany) was used for column chromatography and DC-Alufolien Kieselgel 60 F_{256} plates were used for TLC. MS spectra were recorded on an Applied Biosystems 3200 QTRap spectrometer. HPLC chromatograms were recorded on

Knauer Smartline system equipped with diode array detector. The detection was performed both in 205 nm and 254 nm wavelength. YMC Pack ODS-AQ (150 × 4.6 mm) column with 3 µm particle size was used as stationary phase. A gradient was used from MeCN (65%) to MeCN (80%) in water over 10 min, then to MeCN (98%) over 8 min. with a flow rate of 1.1 mL/min.

4.2. General deprotections:

– Stock solution was made as follows: 0.50 mmol of all $\mathbf{B_i}$ derivatives was measured into a vial and were dissolved in CH_2Cl_2 resulting 100 mL solution. 10 µL of the stock solution was used for HPLC control. 5 mL of the prepared stock solution was used for each deprotections. The 5 mL sample was concentrated and dissolved on the solvent system used for the deprotection procedure.

4.3. *Entry 1:*

5 mL of stock solution was concentrated and the residue was dissolved in the mixture of MeOH (2.5 mL) and toluene (2.5 mL). Thiourea (4 mg) was added to the solution and stirred for 3 h at 80 °C. The volatiles were removed in vacuo and the residue was dissolved in CH_2Cl_2 (20 mL), washed with water (25 mL), dried, filtered and concentrated. The residue was dissolved in CH_2Cl_2 up to 5.00 mL volume and 20 µL was taken for HPLC analysis.

4.4. *Entry 2:*

5 mL of stock solution was concentrated and the residue was dissolved in the mixture of MeOH (2.2 mL) toluene (2.2 mL) and pyridine (0.6 mL). Thiourea (4 mg) was added to the solution and stirred for 3 h at 80 °C. The volatiles were removed in vacuo and the residue was dissolved in CH_2Cl_2 (20 mL), washed with 1N aq. HCl (25 mL), water (25 mL) and sat. $NaHCO_3$ solution (25 mL), dried, filtered and concentrated. The residue was dissolved in CH_2Cl_2 up to 5.00 mL volume and 20 µL was taken for HPLC analysis.

4.5. *Entry 3:*

5 mL of stock solution was concentrated and the residue was dissolved in the mixture of MeOH (0.5 mL) and CH_2Cl_2 (4.5 mL). Hydrazine acetate (20 mg) was added to the solution and stirred for 1 h at rt. The volatiles were removed in vacuo and the residue was dissolved in

CH_2Cl_2 (20 mL), washed with water (25 mL), dried, filtered and concentrated. The residue was dissolved in CH_2Cl_2 up to 5.00 mL volume and 20 µL was taken for HPLC analysis.

4.6. *Entry 4:*

5 mL of stock solution was concentrated and the residue was dissolved in the mixture of MeOH (5 mL) and THF (1 mL). Zn powder (80 mg) and NH_4Cl (40 mg) were added to the solution and stirred for 5 h at rt. The mixture was filtered and the filtrate was concentrated in vacuo and the residue was dissolved in CH_2Cl_2 (20 mL), washed with water (25 mL), dried, filtered and concentrated. The residue was dissolved in CH_2Cl_2 up to 5.00 mL volume and 20 µL was taken for HPLC analysis.

4.7. *Entry 5:*

DBU (50 µL) was added to the 5 mL stock solution and the mixture was stirred for 5 min at rt. The mixture was diluted with CH_2Cl_2 (20 mL), washed with 1N aq. HCl (25 mL), water (25 mL) and sat. $NaHCO_3$ solution (25 mL), dried, filtered and concentrated. The residue was dissolved in CH_2Cl_2 up to 5.00 mL volume and 20 µL was taken for HPLC analysis.

4.8. *Entry 6:*

5 mL of stock solution was concentrated and the residue was dissolved in the mixture of water (1 mL) and THF (5 mL). Camphorsulfonic acid (20 mg) was added to the solution and stirred for 8 h at 50 °C. The mixture was diluted with CH_2Cl_2 (20 mL), washed with water (25 mL) and sat. $NaHCO_3$ solution (25 mL), dried, filtered and concentrated. The residue was dissolved in CH_2Cl_2 up to 5.00 mL volume and 20 µL was taken for HPLC analysis.

4.9. *Entry 7:*

5 mL of stock solution was concentrated and the residue was dissolved in the mixture of water (0.5 mL) and MeCN (4.5 mL). CAN (42 mg) was added to the solution and stirred for 3 h at r.t. The mixture was diluted with CH_2Cl_2 (20 mL), washed with water (25 mL) and sat. $NaHCO_3$ solution (25 mL), dried, filtered and concentrated. The residue was dissolved in CH_2Cl_2 up to 5.00 mL volume and 20 µL was taken for HPLC analysis.

4.10. *Entry 8:*

5 mL of stock solution was concentrated and the residue was dissolved in the mixture of water (0.5 mL) and MeCN (4.5 mL). CAN (70 mg) was added to the solution and stirred for 8 h at r.t. The mixture was diluted with CH_2Cl_2 (20 mL), washed with water (25 mL) and sat. $NaHCO_3$ solution (25 mL), dried, filtered and concentrated. The residue was dissolved in CH_2Cl_2 up to 5.00 mL volume and 20 µL was taken for HPLC analysis.

4.11. *Entry 9:*

5 mL of stock solution was concentrated and the residue was suspended in EtOH (5 mL). $Rh(P(Ph)_3)_3Cl$ (20 mg) was added to the solution and stirred for 3 h at 80 °C. The volatiles were removed in vacuo and the residue was dissolved in the mixture of acetone (4.5 mL) and 1N aq. HCl (0.5 mL) and stirred for 1 h at 60 °C. The volatiles were removed in vacuo and the residue was dissolved in CH_2Cl_2 (20 mL), washed with water (25 mL) and sat. $NaHCO_3$ solution (25 mL), dried, filtered and concentrated. The residue was dissolved in CH_2Cl_2 up to 5.00 mL volume and 20 µL was taken for HPLC analysis.

4.12. *Entry 10:*

Co_2CO_8 (30 mg) was added to the 5 mL of stock solution and stirred for 1 h at r.t.. The volatiles were removed in vacuo and the residue was dissolved in the mixture of MeOH (4 mL), water (0.5 mL) and TFA (0.5 mL). The mixture was stirred for 48 h at 45 °C, then TEA (2 mL) was added and the mixture was filtrated through CELITE. The filtrate was diluted with CH_2Cl_2 (50 mL) and washed with water (2 × 25 mL) and $NaHCO_3$ solution (25 mL) the organic phase was dried ($MgSO_4$), filtrated and concentrated. The residue was dissolved in CH_2Cl_2 up to 5.00 mL volume and 20 µL was taken for HPLC analysis.

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Supplementary data

Supplementary data associated with this article (Experimental procedures, NMR spectra and HPLC chromatograms) can be found, in the online version, at…

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Scheme 1. The compounds used for method development.

Figure1. The principle of the test.

Figure 2. Overlapped HPLC chromatogram of Entry 1 and reference sample.

Figure 3. Overlapped HPLC chromatogram of Entry 3 and reference sample.

Entry	Conditions	**B_1**	**B_2**	**B_3**	**B_4**	**B_5**	**B_6**	**B_7**	**B_8**
1	Thiourea MeOH/tol. reflux	0%	>95%	>95%	>95%	35%	>95%	>95%	>95%
2	Thiourea MeOH/tol./pyr. reflux	0%	>95%	>95%	>95%	>95%	>95%	>95%	>95%
3	H_2NNH_2×AcOH DCM/MeOH	>95%	0%	>95%	>95%	>95%	>95%	>95%	>95%
4	Zn, NH_4Cl MeOH/THF	>90%	>90%	0%	>95%	>95%	>95%	>95%	>95%
5	1% DBU DCM	>95%	>95%	>95%	0%	>95%	>95%	>95%	>95%
6	CSA THF/H_2O	>90%	>90%	>90%	>90%	0%	>90%	>90%	>90%
7	CAN (3 eq.) MeCN/H_2O, 3h	>95%	>95%	>95%	>95%	0%	50%	>95%	>95%
8	CAN (5 eq.) MeCN/H_2O, 8h	>95%	>95%	>95%	>95%	0%	0%	>95%	>95%
9	i: Wilkinson's cat., EtOH ii: 1N HCl, acetone	>95%	>95%	>95%	>95%	0%	>95%	0%	0%
10	i: Co_2CO_8 DCM ii: TFA MeOH/H_2O	50%	70%	20%	95%	0%	90%	50%	0%

TABLE 1. Collected results of different deprotections.