Pergamon

Tetrahedron 55 (1999) 14807–14812

TETRAHEDRON

Monitoring the Solid-Phase Synthesis of Depsides and Depsipeptides. A Color Test for Hydroxyl Groups linked to a Resin.

Oliver Kuisle, Manuel Lolo, Emilio Quiñoá and Ricardo Riguera*

Departamento de Química Orgánica, Facultad de Química, and Instituto de Acuicultura, Universidad de Santiago de Compostela, E-15706 Santiago de Compostela, Spain.

Received 3 August 1999; revised 29 September 1999; accepted 14 October 1999

Abstract: A color test for the detection of hydroxyl groups bonded to a solid support and useful for the control of solid-phase synthesis of depsides and depsipeptides is described. It is carried out directly on the resin and shows a violet to pink color with primary, secondary and tertiary alcohols, and phenols. 16 μmol of free OH groups per g of resin can be easily detected with the naked eye using this procedure. The reaction is based on the formation of the tosylate, its displacement with *p*-nitrobenzylpyridine and conversion of the resin-bound pyridinium salt to a strongly colored internal salt by treatment with base. Amino and carboxyl groups are negative in these conditions. Full loading of Wang and Merrifield resins can be efficiently checked using this test.

Introduction

A critical step in the development of methods for solid-phase synthesis[1] of polymeric and non polymeric substances and in combinatorial chemistry is the assessment of the extent of completion of the reactions carried out on the resin matrix. For this reason, a great deal of effort has been dedicated over the years to find quick and reliable methods for monitoring solid-phase reactions. The desired, but as yet unattained, objective is to develop a method that equals, in terms of simplicity and general availability, the role of TLC in solution phase synthesis.

As a result of this work, several spectroscopic techniques have been successfully adapted to the analysis of synthetic intermediates directly on the resin.[2] Among these, the most powerful and versatile techniques are NMR,[3] using "magic angle spinning" pulse sequences, IR[2] and MS.[4] A more direct but time consuming alternative is cleavage of an aliquot of the resin-bound product and analysis by conventional analytical methods in solution.

The need for a simple procedure for monitoring is especially important in the case of repetitive solid-phase synthesis amenable to automation in the preparation of oligomeric compounds. It is in this context that the ninhydrin test[5] derives all its relevance. The reliability and simplicity of this test has contributed considerably to the development of solid-phase synthesis into a routine tool for the preparation of peptides.

Depsipeptides and depsides are oligomers that are similar to peptides, but in which some or all of the α-amino acids are replaced by hydroxy acids and the peptidic bonds replaced by ester bonds. Therefore, monitoring of the extent of the completion of formation of the ester bond is necessary in this case.

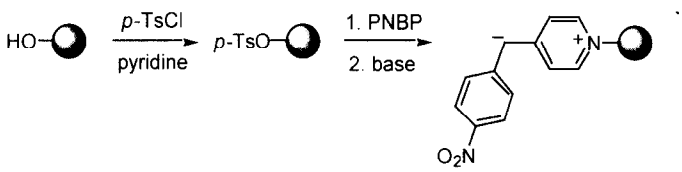
We have recently described a general methodology for the automatic solid-phase synthesis of depsides and

0040-4020/99/$ - see front matter
PII: S0040-4020(99)00945-X

depsipeptides,[6] including analogs of valinomycin. In this article we wish to communicate the experimental details, scope and limitations of the solid-phase test we used to detect the presence of alcohol groups directly on the resin or in the resin-bound growing chain and to determine the completion of the ester coupling step.

Results and Discussion

This test is based[7] on the transformation of the alcohol group into its tosylate, its displacement by *p*-nitrobenzylpyridine (PNBP) and conversion of the solid supported pyridinium salt to a strongly colored internal salt by treatment with base (Scheme 1).



Scheme 1

All the operations can be carried out directly on the resin, they take only a few minutes, and consist of the treatment of a few beads (approx. 1 mg dry resin weight), previously spotted onto an aluminium-backed TLC plate, with solutions of *p*-TsCl and PNBP in toluene and heating with a heat gun. Addition of a solution of piperidine and gentle drying with a heat gun produces a blue to purple color if alcoholic OH groups are present in the resin. For comparative purposes, two resin references representing a positive and a negative blank are also spotted on the TLC plate and the test is simultaneously carried out on the three resin spots. The negative blank is provided by beads of the same resin where the free OH groups have previously been protected (i.e. fully acetylated Wang resin), and the positive blank by a resin known to contain free alcoholic OH groups (i.e. Wang resin or a resin bearing an OH deprotected depside chain[6]). In this way, positive and negative results can be safely associated with the presence or absence of alcohol groups on the matrix.[8]

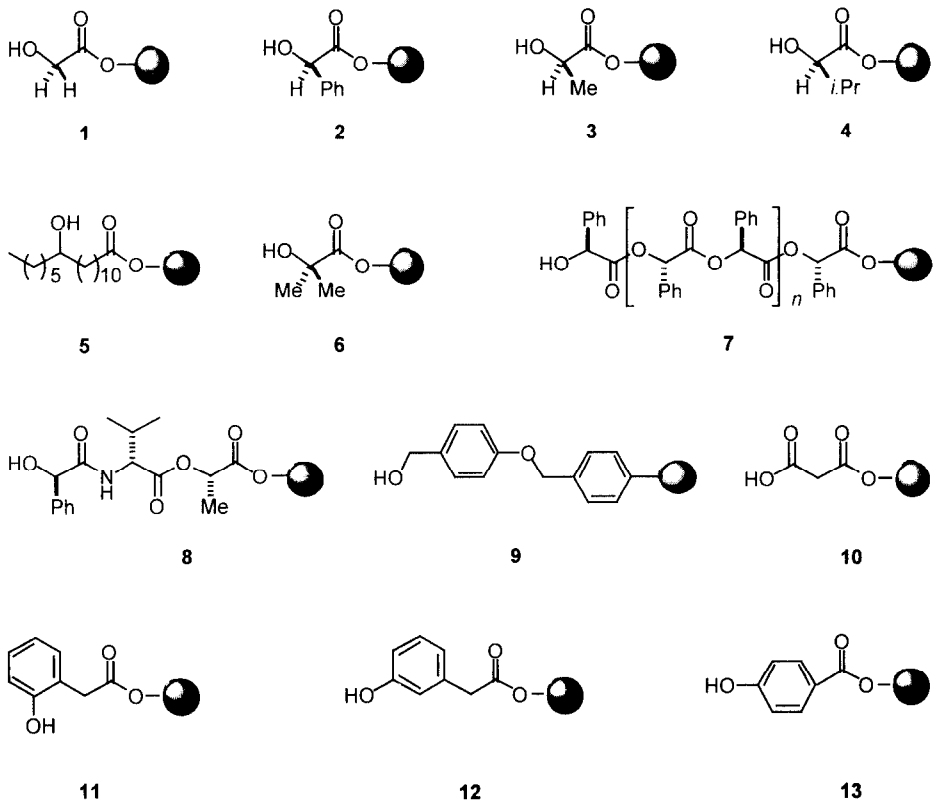
Scope and Sensitivity. In full accordance with the mechanism of the reaction, the test was found to give a positive result for all types of Wang resin-bound alcohols that can react through nucleophilic substitution. The resin-bound primary, secondary and tertiary alcohols **1-6** all produced positive results when submitted to this test and negative if protected as THP-ethers. In addition, positive tests have been obtained for the large series of resin-bound depsides and depsipeptides described in a previous paper[6] (i.e. depside **7** and depsipeptide **8**), which contain free alcohol groups, and negative tests for those in which the OH groups are protected or involved in an ester bond. This test therefore allows quick and simple assessment of the coupling and deprotection steps of the solid-phase synthesis of depsides and depsipeptides. The test is also positive for the benzylic alcohol group characteristic of Wang resin itself (**9**), and negative when all those OH groups have been substituted, thus allowing a rapid test for full loading in the first step of a solid-phase synthesis.

As expected from the mechanism and previous observations,[7] this test can also be used with resins carrying substrates other than alcohols that are able to undergo nucleophilic substitution. In those cases, the tosylation

step is not necessary. Thus, Merrifield resin gives a positive test due to the replacement of chlorine and full loading of this resin with a substrate can be checked by the absence of color.

Negative tests were obtained for resin-bound carboxylic acids such as **10** and for the terminal amino group of peptides and depsipeptides.[6] It has been reported that phenols such as resorcinol and 2-naphthol gave negative results when sprayed with the reagent on TLC plates,[7] and in fact we have confirmed this finding with 4-hydroxybenzoic acid methyl ester and 2- and 3-hydroxyphenylacetic acid methyl ester. When the corresponding free acids of the same compounds were linked to Wang resin (4-hydroxybenzoic acid linked to Merrifield resin was also tested) through the carboxylic group (compounds **11-13**) and the test carried out as described in this paper, all the three resin-bound phenols produced the same color as alcohols **1-6**. If the tosylation step is supressed, no color appears, suggesting that the presence of a good leaving group is necessary for the generation of the chromophore.

As expected, when phenols **11-13** are protected as THP ethers, no such color is observed. Thus, when the test is to be used for monitoring consumption of the alcohol group during a solid-phase synthesis of a depside or depsipeptide, phenolic groups should be previously protected.



In order to establish the limit of sensitivity of this test, samples of Wang resin containing different amounts of alcoholic OH groups were tested. To this end, five aliquots of resin were fully loaded with L-mandelic acid

and submitted to coupling with different amounts (from 0.8 to 4.0 equivalents, Table 1) of O^{α}-THP-protected L-mandelic acid. This led to incomplete couplings and produced resin samples A–E, in which a fraction (x%) of the initial mandelic acid units remain unreacted (free OH), while the rest (100 – x%) have been coupled with the second unit and carry a depside chain with no free OH groups. The exact percentage of OH groups in each sample A–E was obtained from the ratio of L-mandelic acid methyl ester to L-mandelyl-L-mandelic acid methyl ester, determined by GC, after cleavage and treatment with diazomethane, and these results are shown in Table 1.

Samples A–E were submitted to the test as described earlier; a positive result (with a violet color) similar to that obtained with the positive blank was obtained in the cases of resins A and B. For resin samples C and D, the color was pink and less intense, but could still be clearly distinguished by the naked eye from the negative blank. The color of sample E could not be distinguished from that of the negative reference. Since sample D contained 2% of free OH groups, we can fix the lower limit of detection at this percentage, which corresponds to about 16 μmol OH/g resin, based on an initial resin loading of 0.8 mmol OH/g resin.

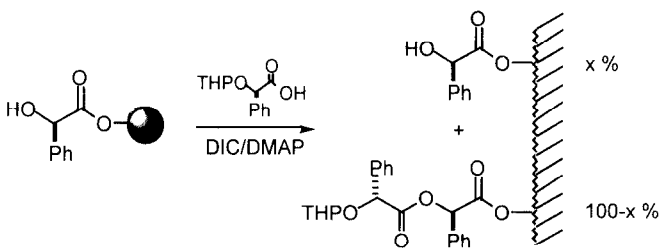


Table 1. Assessment of the test sensitivity with resin samples containing different amounts of free alcohol groups.

sample[a]	eq. of O^{α}-THP-mandelic acid	x%[b]	color
A	0.8	32%	violet
B	1.0	21%	violet
C	1.6	5%	pink
D	2.5	2%	slightly pink
E	4.0	< 2%	undetectable

[a] For preparation of samples see Experimental Section; [b] The percentage of free OH groups was determined by GC (see Experimental Section).

In conclusion, we present here a quick and reliable color test for the detection of resin bound free OH groups of alcohols and phenols. It has been successfully applied for monitoring the solid-phase synthesis of several α-hydroxy ester depsides where the total substitution of the free alcohol group in the growing chain had to be assured before the next coupling step. Similarly, we have used it, in combination with the ninhydrin test,

in the synthesis of depsipeptides where both free amino and hydroxyl groups are the reactive groups. In addition, the test can also be used to assure full loading of the resin with the starting unit (i.e. amino acid, hydroxy acid) of a solid-phase synthesis provided the functional group of the linker can be displaced by nucleophilic substitution.

Experimental Section

Preparation of resin samples A–E. 1 g of Wang resin (0.8 mmol/g loading level) was placed into a 50 mL reaction vessel of an ACT90 peptide synthesizer and submitted to the following reactions: (1) two consecutive depside couplings with O^{α}-THP-L-mandelic acid (710 mg, 3 mmol), DIC (470 μL, 3 mmol) and DMAP (12 mg, 0.1 mmol) in 10 mL of THF for 2 h; washings with CH_2Cl_2, acetone, CH_2Cl_2 (3 x 3 min each); (2) acetylation of the remaining uncoupled OH groups with Ac_2O (0.94 mL, 10 mmol) and DMAP (12 mg, 0.1 mmol) in CH_2Cl_2/pyridine (9:1); (3) cleavage of the THP group with 10 mL of a solution of *p*-TsOH (3 mg/mL) in CH_2Cl_2/MeOH (97:3) (2 x 1 h), preceded by a 3 min washing with the same solution, and followed by washings with CH_2Cl_2, acetone, and CH_2Cl_2 (3 x 3 min each). The resin was allowed to dry and divided into five aliquots (samples A–E) of equal weight. The resin samples were submitted to depside coupling (2 mL THF, 3 mg DMAP), followed by washings as described above, with the following amounts of O^{α}-THP-L-mandelic acid (**1**) and DIC: Sample A: **1** (30 mg, 0.8 eq), DIC (20 μL, 1.2 eq); Sample B: **1** (38 mg, 1.0 eq), DIC (25 μL, 1.5 eq); Sample C: **1** (60 mg, 1.6 eq), DIC (40 μL, 2.4 eq); Sample D: **1** (94 mg, 2.5 eq), DIC (63 μL, 3.8 eq); Sample E: **1** (150 mg, 4 eq), DIC (100 μL, 6 eq).

Quantification of the free OH groups in resin samples A-E by GC. 30 mg each of resins A-E were treated with 1:1 TFA/CH_2Cl_2 at rt for 1 h (no cleavage of the depside bond is produced under these conditions; see ref. 6). The beads were filtered off, the solution was evaporated several times with CH_2Cl_2 and the residue methylated with diazomethane in ether (2 h). The resulting mixture was dissolved in 0.5 ml of CH_2Cl_2 and 1 μl of this solution was analyzed by GC (Ultra-1, 25m x 0.32mm x 0.52 μm; 170°C, 4 min - 5°C/min - 210°C). The area ratios of L-mandelic acid methyl ester (t_R = 4.22 min) and L-mandelyl-L-mandelic acid methyl ester (t_R = 24.23 min) are shown in Table 1.

Experimental procedure for the solid-phase test for alcohols: One drop of a suspension of resin beads in CH_2Cl_2 (approx. 1 mg dry resin) was taken directly from the reaction vessel with a Pasteur pipette and placed on a silica gel TLC plate. Two reference samples were placed on the same plate using the same procedure: a positive blank formed by an OH-bearing resin (i.e. Wang resin or a resin with a completely deprotected depside chain) and a negative blank (i.e. beads of fully acetylated Wang resin). The samples were spread out to a thin circular film of about 0.5 cm diameter by carefully dropping CH_2Cl_2 from a Pasteur pipette.[9] The three spots were then successively treated in the following way: (1st) 2 drops of a toluene solution containing 0.03 M *p*-TsCl were added; (2nd) 2 drops of a solution of 0.075 M 4-*p*-nitrobenzylpyridine in toluene were added and the plate was then heated from underneath with a heat gun[10] until the orange color that initially develops had disappeared completely (about 10-12 seconds); (3rd) 2 drops of a 10% piperidine solution in $CHCl_3$ were added to each spot followed by gentle drying of the plate with a heat gun; (4th) the three samples were carefully washed with several drops of CH_2Cl_2 and allowed to dry.

If no free OH are present, the test sample should appear colorless as does the negative blank. If color

appears, unreacted OH groups are present in the resin (see Table 1). Since the white silica "background" often remains colored to a certain extent in spite of the washings, an alternative is to scrape the dry resin beads from the TLC plate and to deposit them onto a white well plate. This enhances the contrast and allows easier distinction of positive and negative test results.

The solutions of PNBP and *p*-TsCl can be stored at 4 °C for a few weeks only; after prolonged storage periods they lose their efficacy.

Acknowledgement. This work was supported by grants from CICYT (PM98-0227) and XUGA (20910B96 and 20908B97).

References and Notes

1. Brown, A. R.; Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees. D. C. *Synlett* **1998**, 817-827.
2. Yan, B *Acc. Chem. Res.* **1998**, *31*, 621-630.
3. Riedl, R.; Tappe, R.; Berkessel, A. *J. Am. Chem. Soc.* **1998**, *120*, 8994-9000.
4. Egner, B. J.; Bradley, M. *Tetrahedron* **1997**, *53*, 14021-14030.
5. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595-598.
6. Kuisle, O.; Quiñoá, E.; Riguera, R. *J. Org. Chem.* **1999**, in press.
7. The same reaction has been used for the detection of alcohols in paper chromatography: Pomonis, J. G.; Severson, R. F.; Freeman, P. J. *J.Chromatog.* **1969**, *40*, 78-84.
8. We also tried to carry out the test with a suspension of the resin in a test tube but the results are far better when the resin is "fixed" on the silica and the test directly carried out on the TLC plate. The reason for this is that only the resin beads show the characteristic coloration; the solution has a different color, which makes it difficult to distinguish the color of the resin.
9. When the resin suspension is spotted on the TLC plate, it usually forms a small heap that should be flattened with drops of CH_2Cl_2. The contact with the support is better and the color of the resin beads more homogeneous when the sample is spread out to give a thin film.
10. We used a heat gun (1600 W) with a maximum temperature of 530°C as indicated on the gun. The temperature of the heated TLC plates was determined with a K-type thermocouple and found to be aprox. 280°C on the upper side containing the spots and aprox. 400°C on the lower side.