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Microwave-induced organic reactions of bile acids: Esterification, deformylation and deacetylation using mild reagents

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An efficient and convenient procedure for the esterification, deformylation, and deacetylation of bile acids is described. This is achieved by the addition of a catalytic amount of methanesulfonic acid or para-*toluene sulfonic acid to a solution of bile acid in methanol in the domestic microwave oven. All these reactions were completed in the microwave oven within 1–3 min at 60% power (390 W) and the desired bile acids, namely trihydroxy-5β-cholestanoic acid, (23R)-3α,7α,23-trihydroxy-5β-cholan-24-oic acid, ursocholic acid and 7-ketolithocholic acid were isolated in 86–94% yield. (Steroids* **60**:453–457, 1995)

Keywords: microwave; bile acids; esterification; deformylation; deacetylation; methanesulfonic acid/methanol; para-toluene sulfonic acid/methanol

Introduction

Recently there has been increasing interest in the use of microwave irradiation techniques in organic synthesis.[1–4] A number of simple synthetically useful organic reactions have been carried out in the microwave oven in sealed vessels.[3,4] With such microwave irradiation techniques, remarkable rate enhancements have been observed and, in some cases, cleaner reactions with easier workup than compared to conventional heating methods.[3–9]

Recently many laboratories including our own have described microwave-induced reactions in open vessels in unmodified microwave ovens.[5–9] In each case, the reactions proceeded in a highly accelerated manner and the yields and purity of the final products were comparable with the traditional protocol.

In the present paper we describe several esterification reactions of bile acids employing a commercial microwave oven using mild reagents methanesulfonic acid (MSA) or *para*-toluene sulfonic acid (PTS) in methanol. In addition, deprotection reactions of acetylated and performylated bile acids in the presence of catalytic amount of MSA or neat PTS are also described. In these experiments it was observed that MSA was as effective an acid as HCl, H_2SO_4 or PTS, for the esterification of bile acids (Figure 1) and was generally preferred to these strong acids because, being a mild acid, it was less damaging to reactants.

Experimental

Melting points

Melting points were determined on a Thermolyne apparatus (Thermolyne Corp., Dubuque, IA, USA) model MP-12600 and are uncorrected. MSA was a generous gift from Atochem Organic Chemicals (Edison, NJ, USA) and was used without purification.

Thin-layer chromatography (TLC)

All trihydroxy bile acid methyl esters and their corresponding acids were separated on Silica gel G plates (Analtech, Uniplates, Newark, NJ, USA; 0.25 mm thickness) in the solvent system: $CHCl_3/(CH_3)_2CO/CH_3OH$, 70:50:5 (v/v/v). For dihydroxy bile acids and their corresponding esters, the solvent system $CHCl_3/(CH_3)_2CO/CH_3OH$, 70:50:3.5 (v/v/v) was used. Bile acids were visualized by spraying the plates with a solution of phosphomolybdic acid in propanol-2 (3.5%) followed by 10% sulfuric acid.

Gas-liquid chromatography

Capillary GLC analysis of bile acid methyl esters (as their trimethylsilyl derivatives) was performed on Hewlett-Packard model No. 4890 (equipped with a flame ionization detector) and a split-

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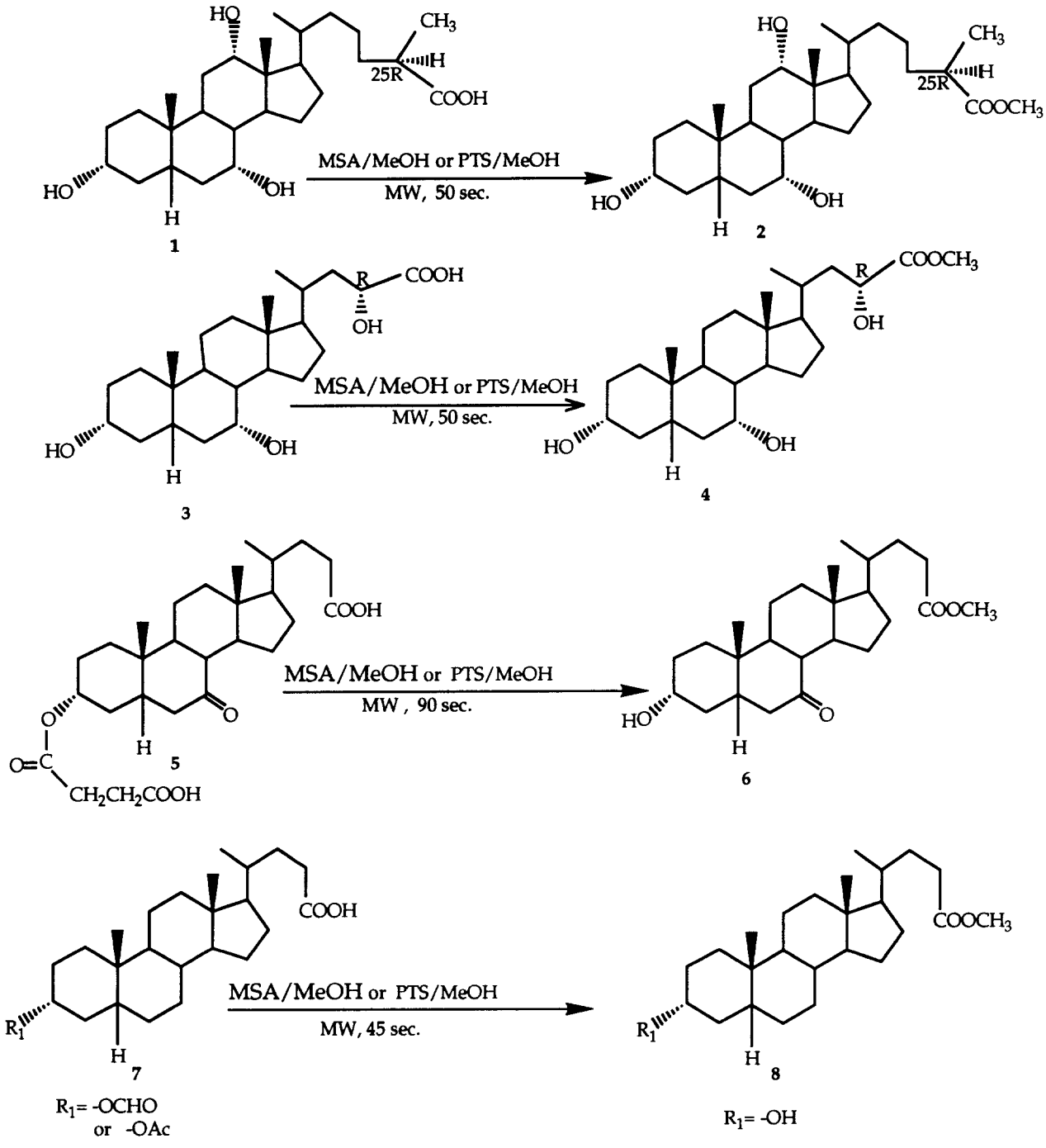

Figure 1 **1.** (25*R*)-3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid. **2.** (25*R*)-methyl 3α,7α,12α-trihydroxy-5β-cholestan-26-oate. **3.** (23*R*)-3α,7α,23-trihydroxy-5β-cholan-24-oic acid. **4.** (23*R*)-methyl-3α,7α,23-trihydroxy-5β-cholan-24-oate. **5.** 3α-succinoxy-7-oxo-5β-cholan-24-oate. **6.** 3α-hydroxy-7-oxo-5β-cholan-24-oate. **7.** 3α-acetoxy (or 3α-formyloxy)-5β-cholan-24-oic acid. **8.** Lithocholic acid methyl ester.

column injector using a CP Sil 5 (CB) WCOT capillary column (25 m × 0.22 mm with 0.13-mm film thickness). Helium was used as a carrier gas at a flow rate of 20.2 mL/min (135 kPa).

The microwave oven used in these experiments was a domestic Whirlpool Commercial Microwave model number 3600XS operating at 2450 MHZ (total cooking power of the microwave oven = 650 W). The reactions described in this study were carried out in Erlenmeyer flasks, or scintillation vials covered with a funnel, or watch glass and were found to be more convenient and were used in these microwave-induced reactions. Alternatively, some of the reactions were also carried out in sealed 5 mL or 10 mL Teflon vessels for comparison as described previously.[7]

Cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid), ursoholic acid (3α,7β,12α-trihydroxy-5β-cholan-24-oic acid), chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid), ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid), deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic-acid), lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid) were purchased from Sigma. Other unusual bile acids (Figure 1, Compounds 1, 3, and 5) which were utilized in the microwave

irradiation experiments were either synthesized or isolated and characterized as previously described. Briefly, (25*R*)-3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (Figure 1, Compound 1) was isolated from alligator's bile as described by Tint et al.[10] (23*R*)-3α,7α,23-Trihydroxy-5β-cholan-24-oic acid (β-phocecholic acid, Figure 1, Compound 3) was isolated from duck's bile and characterized as described previously.[7,11] 3-Hemisuccinate of 7-ketolithocholic acid (compound 5, Figure 1) was synthesized, purified, and characterized as described by Yoshi et al.[12]

Experimental and results

All bile acids, namely cholic, ursocholic, chenodeoxycholic, ursodeoxycholic, deoxycholic, and lithocholic acid were esterified in a commercial microwave oven using the mild reagent methanesulfonic acid or *para*-toluene sulfonic acid in methanol. All of these reactions provided the corresponding methyl esters in 90–95% yield. Other unusual bile acids, compounds 1, 3, and 5, illustrated in Figure 1, also yielded the methyl esters (compounds 2, 4, and 6 Figure 1) in excellent yield after esterification with these reagents. In addition, simultaneous cleavage of 3-hemisuccinates, acetates, and formates (compounds 5 and 7, Figure 1, to compounds 6 and 8, Figure 1) was also accomplished.

Some representative general procedures, such as esterification, formylation, deformylation and deacetylation for bile acids are listed below.

Formation of 7-Ketolithocholic acid methyl ester via 3-hemisuccinate of 7-ketolithocholic acid (compound 5, Figure 1)

A solution of 100 mg of 3-hemisuccinate of 7-ketolithocholic acid in 10 mL of MeOH was treated with 6 drops of 4 M MSA and the predigested mixture was irradiated in the microwave oven in an Erlenmeyer flask for 90 seconds at 60% power (390 W, the total cooking power of the microwave oven = 650 W). Completion of the reaction was monitored by thin-layer chromotography (TLC) and gas-liquid chromatography (GLC). Usually there was a quantitative conversion of bile acid methyl esters in 50–60 seconds. After the heating was completed, the flask was cooled to room temperature and the solution was poured into 200 mL of ice cold water with stirring. The precipitate was collected, washed with water, and dried to yield 95 mg (95%) of 7-ketolithocholic acid methyl ester (TLC, R_f = 0.59 and R_f = 0.36 of 3-hemisuccinate of 7-ketolithocholic acid, solvent system: $CHCl_3$:CH_3COCH_3:CH_3OH 70:20:1.0, v/v/v) and was shown to be identical in all respects (i.e., TLC, ^1H NMR, MS, and m.p.) as previously described (7,15). Alternatively, when the reaction was repeated with PTS/MeOH reagent (100 mg substrate, 20 mg PTS, 8 mL MeOH), the esterification and removal of hemisuccinate also occurred simultaneously, as observed with MSA/MeOH (compound 6, Figure 1). However, PTS-catalyzed esterification and deprotection reactions required the use of a base to neutralize the excess acid and organic solvents for extracting the final reaction mixture. All the methyl esters prepared in the microwave oven by the PTS/MeOH reagent were identical to the ones prepared at room temperature as previously described by us[13] and were purified by flash chromatography or recrystallization.[14]

Similar protocol on 3-acetoxy (or 3-formyloxy) lithocholic acid (compound 7, Figure 1) provided methyl lithocholate (compound 8, Figure 1) in 98% yield with MSA/MeOH reagent and 90% yield with PTS/MeOH.

Microwave-induced preparation of (triformyloxyursocholic acid, Figure 2, compound 2)

A solution of ursocholic acid, (3α,7β,12α-triformyloxy-5β-cholan-24-oic acid (1 g), 10 mL formic acid (96%), 0.2 mL perchloric acid was irradiated in the microwave oven in an Erlenmeyer flask covered with a funnel for 2 min at 60% power.[7] The product, triformyloxyursocholic acid was formed in almost quantitative yield (98%) as monitored by TLC and was shown to be identical in all respects (i.e., TLC, ^1H NMR, IR, MS, and m.p.) as previously described.[15]

Deformylation and simultaneous esterification of 3α,7β,12α-triformyloxy-5β-cholan-24-oic acid to ursocholic acid methyl ester; Figure 2, Compound 3)

To a solution of 100 mg of triformylated ursocholic acid (Figure 2, Compound 2) in 6 mL MeOH was added 5 drops of MSA and the reaction mixture was irradiated for 2 min in a commercial microwave oven. After usual workup, 92 mg of methylursocholate was obtained. Similar protocol with PTS/MeOH (PTS 20 mg) provided methylursocholate (yield 82%).

Selective hydrolysis of the 3α-acetate and 3α,7α-diacetate functional groups of cholic acid derivatives (Figure 2, compound 5)

Methyl 3α,7α,12α-triacetoxy-5β-cholan-24-oate (Figure 2, compound 5) was prepared in the microwave oven (Ac_2O, pyr., catalytic DMAP, 1 min, 60% power) as previously described.[7] 20 mg of compound 5 was treated with the reagents MSA/MeOH (50:1, v/v) or PTS/MeOH, 5 mg/2 mL) and after shaking was microwaved for 1 min at 60% power. After cooling and usual work up it resulted in a 1:1 (v/v) mixture of 3α-hydroxy,7,12-diacetoxymethylcholate and 12α-acetoxy methyl cholate (compounds 6 and 7, Figure 2). Compound 5 when heated in the microwave for 3 minutes and reaction mixture monitored by TLC gave 12α-acetoxy methyl cholate (compound 7, Figure 2) in 88% yield with MSA/MeOH and a little less yield (~75%) when PTS/MeOH was used. TLC indicated that the higher R_f value of the acetate reactant changed to the lower R_f value of the alcohol product.

Discussion

In the present studies we have described esterification and deprotection reactions of bile acids in 5–100 mg scale in polar organic solvents in a commercial microwave oven at low power settings. This fast technique provided bile acid methyl esters and their deprotected derivatives (Figure 1 and Figure 2) comparable or better than those obtained by the conventional heating methods.[13,15,16] In addition, in each case the dependence of reaction yield on time, the original diastereomeric ratios, and the absolute stereochemistries of the products (Figure 1, compounds 1 and 3) was preserved by the microwave heating mode. Furthermore, the new procedure offers operational simplicity and does not cause discoloration or byproduct formation. Since MSA and PTS[13] are milder catalytic reagents, they do not promote ether formation or cause the formation of peroxides, thus eliminating the use of diazomethane which can be extremely toxic and hazardous when preparing large amounts of bile acid methyl esters. Since MSA is an inexpensive reagent and is water soluble, it can be washed out with H_2O, avoiding the use of strong bases (e.g., NaOH, KOH, K_2CO_3) which can destroy the labile functional groups in the molecule.

Methanesulfonic acid has been used in the past as a

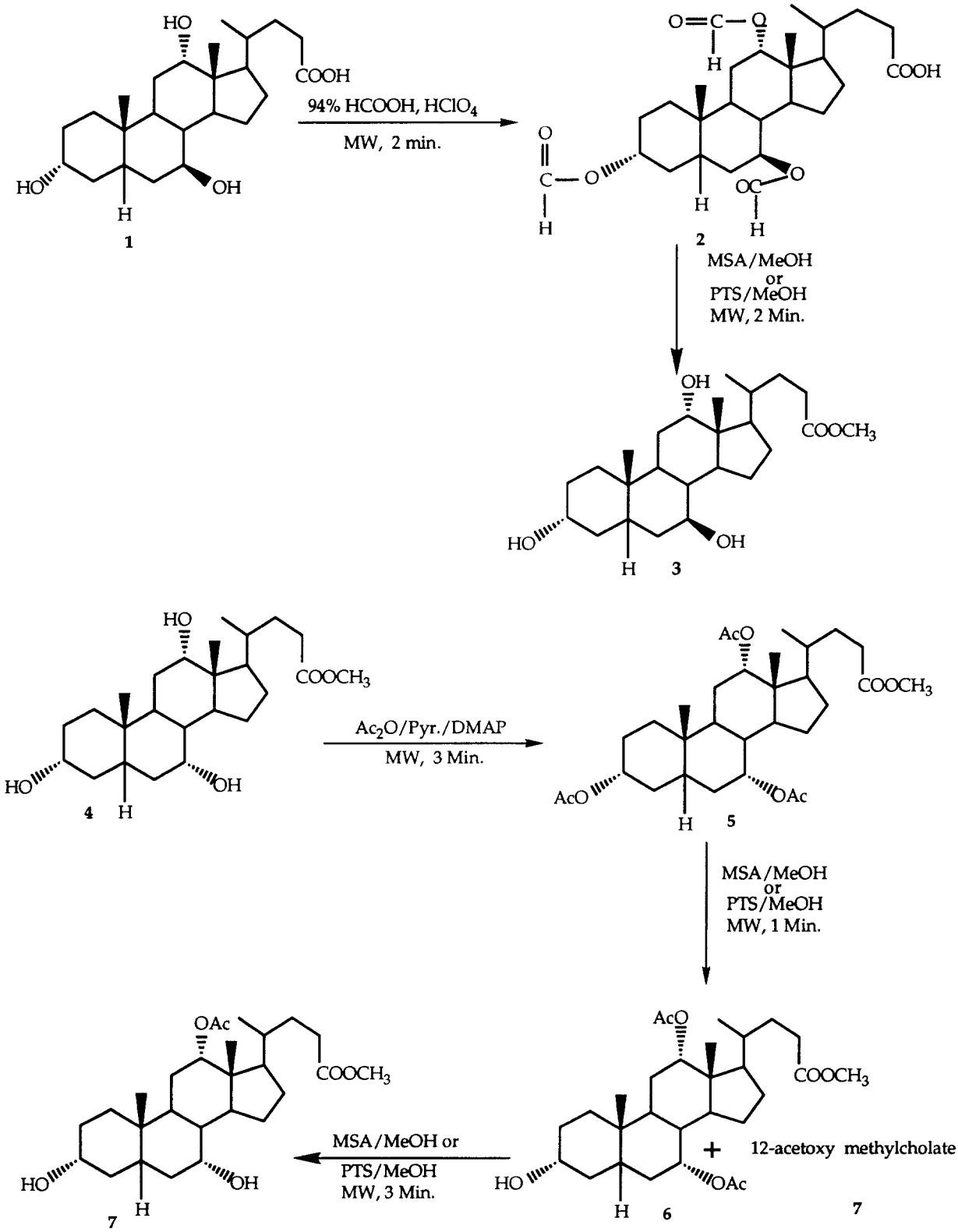

Figure 2 **1.** 3α,7β,12α-trihydroxy-5β-cholan-24-oic acid. **2.** 3α,7β,12α-triformyloxy-5β-cholan-24-oic acid. **3.** methyl 3α,7β,12α-trihydroxy-5β-cholan-24-oate. **4.** Methyl cholate. **5.** methyl 3α,7α,12α-triacetoxy-5β-cholan-24-oate. **6.** 3α-hydroxy-7,12 diacetoxy methyl cholate. **7, 8.** 12α-acetoxy methyl cholate.

solvent and as a catalyst for the conversion of carboxylic acids into peroxy acids.[17] Furthermore, its use in catalytic amounts for the hydrolysis of esters under nonalkaline conditions (0.1 mol ester, 0.1 mol MSA, 100 mL 90% HCOOH, reflux ×5 h) has been reported to yield acids in 64–97% yield.[18] The results in comparison to other acids showed that MSA was the acid of choice as H_2SO_4 gave much poorer yields, PTS still lower, and CF_3COOH and H_3PO_4 almost none.[18]

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Notes and abbreviations

NMR	nuclear magnetic resonance
GLC	gas-liquid chromatography
RRT	relative retention time
TLC	thin-layer chromatography
DMAP	4-N,N-dimethyl aminopyridine
MSA	methanesulfonic acid
PTS	*para*-toluene sulfonic acid
MW	microwave oven
Ac_2O	acetic anhydride
pyr.	pyridine

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