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Large-Scale Synthesis of Immunoactivating Natural Product, Pristane, by Continuous Microfluidic Dehydration as the Key Step

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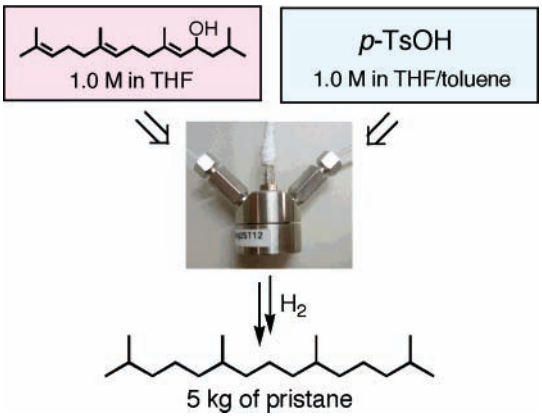
ABSTRACT



An efficient protocol of dehydration was developed under microfluidic conditions. The method was applied to a multikilogram synthesis of pristane, a biologically important natural product, which is now widely used as an adjuvant for monoclonal antibody production.

2,6,10,14-Tetramethylpentadecane (pristane) is a saturated isoprenoid isolated from the basking shark, *Cetorhinus maximus*.[1] This hydrocarbon oil is known to induce tumorogenesis in mice and arthritis and lupus nephritis in rats, and has been widely used as an adjuvant for monoclonal antibody production in mouse ascites.[2] However, in 2002, the basking shark was listed in Article II of the Washington Convention (Convention on International Trade in Endangered Species of Wild Fauna and Flora),[3] and since then, the availability of pristane from natural sources has been very limited.[1] Therefore, an efficient chemical synthesis of pristane has been long desired.

When considering the nonstereoselective synthesis of this simple hydrocarbon, one can immediately devise a straightforward route, i.e., (1) oxidation of farnesol **1**, (2) alkylation,

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(1) (a) Bigelow, H. B.; Schroeder, W. C. *I. Sharks Mem. Sears Found. Mar. Res.* **1948**, 189−195. (b) Matthews, L. H. *Philos. Trans. R. Soc. London, Ser. B* **1950**, *234*, 247−316. (c) Parker, H. W.; Stott, F. C. *Zool. Meded.* **1965**, *40*, 305−319. (d) Compagno, L. J. V. *FAO Fish. Synopsis* **1984**, *4*, 233−236. (e) Clark, E. *Natl. Geographic* **1992**, *182*, 120−138.

(2) (a) Satoh, M.; Richards, H. B.; Shaheen, V. M.; Yoshida, H.; Naim, M. J. O.; Wooley, P. H.; Reeves, W. H. *Clin. Exp. Immunol.* **2000**, *121*, 399−405. (b) Holmdahl, R.; Lorentzen, J. C.; Lu, S.; Olofsson, P.; Wester, L.; Holmberg, J.; Pettersson, U. *Immunol. Rev.* **2001**, *184*, 184−202. (c) Gado, K.; Silva, S.; Paloczi, K.; Domjan, G.; Falus, A. *Haematologica* **2001**, *86*, 227−236.

(3) Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES): http://www.cites.org/index.html.

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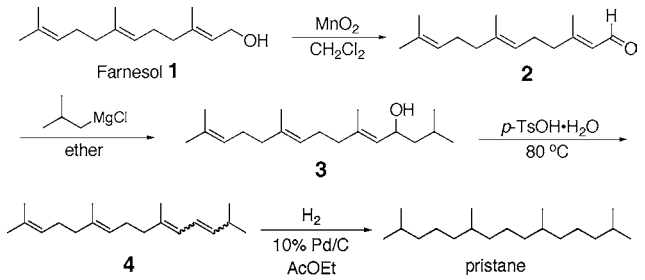
(3) dehydration, and (4) hydrogenation, as shown in Scheme 1. Small quantities (50 mg) of the sample are readily prepared

Scheme 1. Synthesis of Pristane



via this route; however, synthesis of the required 200 kg annually at greater than 98% purity presents unique challenges. In particular, the preparation of 5 kg of pristane per week is necessary to supply enough material to the market.

The challenging step in Scheme 1 is the acid-catalyzed dehydration of allylic alcohol **3**. When the reaction was performed on a 100 mg scale with a catalytic amount of *p*-TsOH in benzene at 80 °C, the corresponding diene **4** was obtained in 55% yield as a mixture of (*E*)- and (*Z*)-stereoisomers, which was transformed to pristane by hydrogenation. However, when the scale was increased to 100 g, various cation-mediated byproducts, such as cyclized products or alkyl group-migrated compounds, were produced. As expected, these hydrocarbons were very difficult to separate from the desired diene **4** (yield estimated to be less than 20%), even by repeated distillation or by silica gel chromatography, although the latter is not realistic for kilogram-scale purification. A more direct olefination with use of the Wittig reaction with aldehyde **2** cannot be used either, since the triphenylphosphine oxide generated is problematic for such a large-scale synthesis. In this paper, we report the successful application of a continuous microfluidic system to the dehydration reaction, which led to the 5-kg-scale synthesis of pristane performed only by one chemist within a week.

A continuous flow microreactor, which is reported to realize efficient mixing and fast heat transfer, is recognized as an innovative technology in recent organic syntheses.[4,5] The flow system also allows the reaction to be quenched immediately after the formation of an unstable product, such as diene **4**, under acidic conditions; hence the method is well-suited for the present large-scale dehydration of **3**. We have recently applied a microfluidic system to other cation-mediated reactions and improvements have been realized for glycosylation reaction[6] and the reductive opening of benzylidene groups.[7]

We examined the microfluidic dehydration under the following conditions (Figure 1): Allylic alcohol **3** (1.0 M in THF) was mixed with a solution of *p*-TsOH (various concentrations of 0.2−1.0 M in THF:toluene 1:1)[8] at 90 °C by using an IMM micromixer[9] at a flow rate of 0.3 mL/min. After the reaction mixture was allowed to flow for an additional 47 s through a reactor tube ($\Phi = 1.0$ mm, $l = 1.0$ m) at 90 °C, the mixture was quenched with a saturated $NaHCO_3$ solution at room temperature. When a 0.2 M solution of *p*-TsOH was used, only a trace amount of **4** was obtained and the starting material **3** was largely recovered. However, we found that the yield of the dehydrated compound depends on the concentration of the acid; **4** was finally obtained in 80% yield (overall, from farnesol **1**) at an acid concentration of 1.0 M. It is noted that under the established microfluidic conditions, the formation of other byproducts could not be detected by TLC analysis.[10]

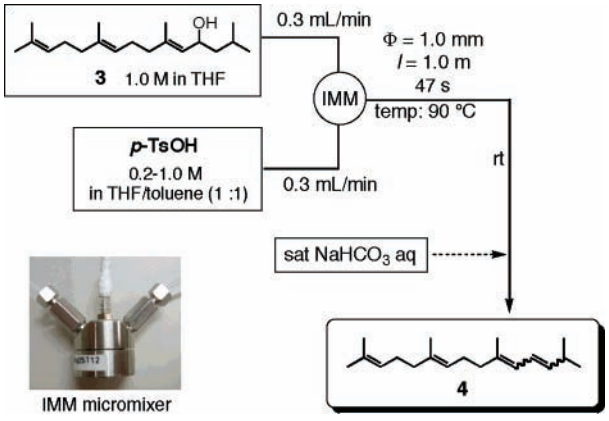


Figure 1. Optimization of microfluidic dehydration.

To evaluate the efficiency of dehydration under the microfluidic conditions, the reactivity of β-hydroxyketone **5** and alkanol **7** was also tested (Scheme 2). Gratifyingly, both substrates provided the corresponding dehydrated products in almost quantitative yields under the conditions established in Scheme 2. Thus, β-hydroxyketone **5** gave (*E*)-unsaturated ketone **6**[11] quantitatively upon mixing with

(4) Representative reviews, see: (a) Ehrfeld, W., Ed. *Microreaction Technology*; Springer: Berlin, Germany, 1998. (b) Manz, A.; Becker, H., Eds. *Mycrosystem Technology in Chemistry and Life Sciences*; Springer: Berlin, Germany, 1998. (c) Ehrfeld, W.; Hessel, V.; Lowe, H. *Microreactors*; Wiley-VCH: Weinheim, Germany, 2000. (d) Hessel, V.; Hardt, S.; Lowe, H. *Chemical Micro Process Engineering*; Wiley-VCH: Weinheim, Germany, 2004. (e) Yoshida, J.-I.; Suga, S.; Nagaki, A. *J. Synth. Org. Chem. Jpn.* **2005**, *63*, 511−522.

(5) Recent applications, see: (a) Pennemann, H.; Hessel, V.; Loewe, H. *Chem. Eng. Sci.* **2004**, *59*, 4789−4794. (b) Jahnisch, K.; Hessel, V.; Loewe, H.; Baerns, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 406−446. (c) Nagaki, A.; Togai, M.; Suga, S.; Aoki, N.; Mae, K.; Yoshida, J.-I. *J. Am. Chem. Soc.* **2005**, *127*, 11666−11675. (d) Ratner, D. M.; Murphy, E. R.; Jhunjhunwala, M.; Snyder, D. A.; Jensen, K. F.; Seeberger, P. H. *Chem. Commun.* **2005**, 578−580.

(6) Fukase, K.; Takashina, M.; Hori, Y.; Tanaka, D.; Tanaka, K.; Kusumoto, S. *Synlett* **2005**, 2342−2346.

(7) Tanaka, K.; Fukase, K. *Synlett.* In press.

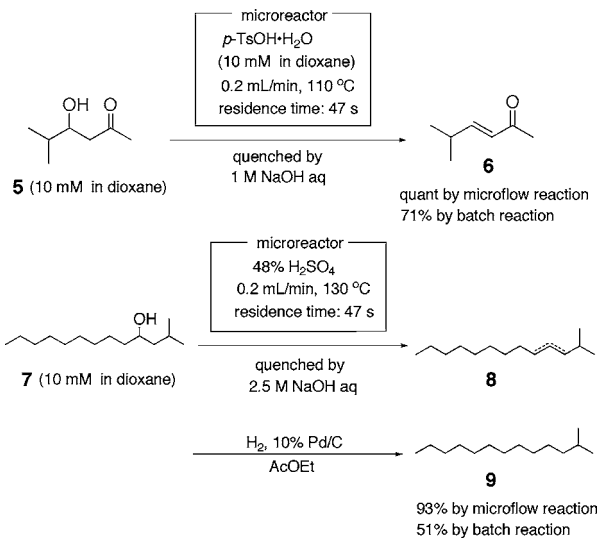
(8) *p*-Toluenesulfonic acid was not completely soluble in toluene. The use of methanol as a cosolvent provided the methoxylated product.

(9) IMM micromixer: http://www.imm-mainz.de/.

(10) We also tested hydrochloric acid or sulfuric acid as more easily used acids for a large-scale synthesis, but the starting material **3** was not consumed completely under these conditions.

(11) Tanaka, K.; Kobayashi, T.; Mori, H.; Katsumura, S. *J. Org. Chem.* **2004**, *69*, 5906−5925.

Scheme 2. Microfluidic Dehydration of β-Hydroxyketone **5** and Alkanol **7**



p-TsOH solution in dioxane at 110 °C. Similarly, the microfluidic mixing of simple alkanol **7** with 48% sulfuric acid at 130 °C gave the alkene **8** as its olefin regioisomeric isomers, which were hydrogenated in the presence of 10% Pd/C under hydrogen atmosphere to provide the hydrocarbon **9** in 93% yield for two steps.[12] For both cases, the conventional batch reaction gave lower yields of the products (71% for **6** and 51% for **9**) due to recovery of the starting materials and formation of other hydrophobic byproducts. Therefore, an efficient and general protocol for dehydration was realized by using the micromixing system.

Having established the optimal conditions for dehydration, the kilogram-scale synthesis of pristane was examined (Scheme 1). About 8 kg of farnesol **1** was treated with 80 kg of MnO_2 to give aldehyde **2**. A large amount of the produced manganese hydroxide was collected and recycled by heating to 200 °C. The crude aldehyde **2** was then reacted with isobutylmagnesium chloride prepared from isobutyl chloride and magnesium tuning,[13] to provide allylic alcohol **3**.

The crude alcohol **3** was subjected to the key microfluidic dehydration under the conditions established in Figure 1. For such a large-scale microfluidic reaction, we introduced a new micromixer, "Comet X-01" (Techno Applications Co., Ltd., Tokyo, Japan),[14] which exhibited similar mixing efficiency to the IMM micromixer for dehydration. "Comet X-01" has been designed to avoid blockages by using a tube with a relatively large inner diameter. This is especially useful when a readily crystallized material, such as TsOH, is used at high concentration. The Comet X-01 mixer is also advantageous when performing the reaction for a long time, because the micromixing device can be tightly connected to either a syringe or an HPLC pumping system, so that solution dripping and leakage is minimal. Therefore, this affordable new device is well suited for establishing a micro chemical plant.

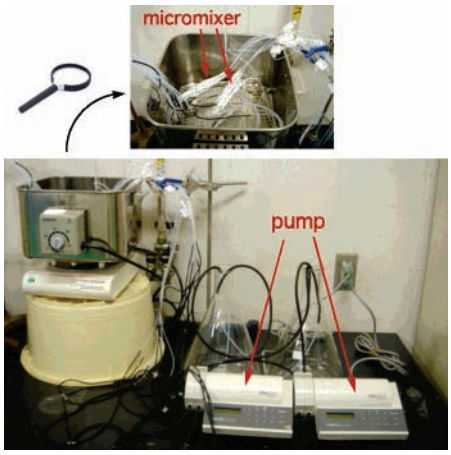


Figure 2. Large-scale microflow system for dehydration.

As shown in Figure 2, we arranged 10 micromixers in parallel and successfully performed an 8-kg-scale dehydration over 3−4 days. It is noted that the present micro chemical plant does not require any special apparatus or devices and that a simple system (Figure 2) continuously performed efficient dehydration. The solution eluted from the micromixing system was quenched by a saturated $NaHCO_3$ solution, extracted with ethyl acetate, and concentrated, and the mixtures were quickly passed through a short silica gel pad to remove the hydrophilic byproducts, affording the pure diene **4**. Finally, hydrogenation in the presence of 10% Pd/C under hydrogen atmosphere provided pristane in 50−55% overall yields (∼5 kg) with >99% purity based on gas chromatographic analysis.[15] The synthetic pristane obtained through this route was confirmed to induce antibody production at the same efficiency as natural pristane.[16]

In summary, we have established an efficient method for dehydration under microfluidic conditions. The present microfluidic protocol is general for β-hydroxyketone, simple alkanol, and allylic alcohol, and the method was successfully applied to a multikilogram synthesis of pristane in one batch. Since the present pristane synthesis involves only one simple

(12) Data for **9**: ^{1}H NMR (500 MHz $CDCl_3$) δ 0.86 (d, 6H, J = 6.7 Hz), 0.88 (t, 3H, J = 6.9 Hz), 1.13−1.16 (m, 2H), 1.26−1.32 (m, 18H), 1.52 (qt, J = 6.6, 6.6 Hz); ^{13}C NMR (125 MHz $CDCl_3$) δ 14.1, 22.66, 22.71, 27.4, 28.0, 29.4, 29.69, 29.73 (overlapped), 30.0, 32.0, 39.1.

(13) Tanaka, K.; Kamatani, M.; Mori, H.; Fujii, S.; Ikeda, K.; Hisada, M.; Itagaki, Y.; Katsumura, S. *Tetrahedron* **1999**, *55*, 1657−1686.

(14) A new micromixing device, "Comet X-01", is available from Techno Applications Co., Ltd., 34-16-204, Hon, Denenchofu, Oota, Tokyo, 145-0072, Japan (e-mail: yukio-matsubara@nifty.com). Comet X-01 exhibited much superior mixing efficiency to the simple T-shape mixer and similar efficiency to the IMM micromixer for acid-catalyzed reactions (ref 7). The detailed structure and mixing system will be reported elsewhere.

(15) Data for the synthetic pristane (mixture of diastereomers): ^{1}H NMR (500 MHz $CDCl_3$) δ 0.78 (d, 6H, J = 6.6 Hz), 0.80 (d, 12H, J = 6.6 Hz), 0.97−1.03 (m, 4H), 1.04−1.09 (m, 4H), 1.13−1.25 (m, 8H), 1.27−1.33 (m, 2H), 1.43−1.48 (m, 4H); ^{13}C NMR (125 MHz $CDCl_3$) δ 19.7, 19.8, 22.6, 22.7, 24.5, 24.79, 24.80, 28.0, 32.78, 32.80, 37.3, 37.39, 37.41, 37.5, 39.4.

(16) Available from Kishida Chemical Co., Ltd. (No. 980-60544 and 980-60545, Sept 13, 2006); http://www.kishida.co.jp/.

purification step by filtration with a silica gel pad, we believe that our protocol is superior to the previously known synthesis, purification of which necessitates tedious repetitive distillation at the final stage.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR of **9** and pristane. This material is available free of charge via the Internet at http://pubs.acs.org.

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