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Efficient chemoenzymatic enantioselective synthesis of diacylglycerols (DAG)

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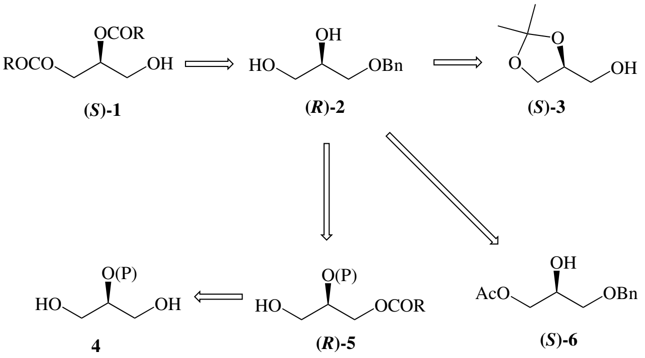
Abstract—A new efficient chemoenzymatic methodology for the production of 3-*O*-benzyl-*sn*-glycerol and 1,2-*O*-dipalmitoyl-*sn*-glycerol has been developed. It starts from racemic 1-*O*-benzylglycerol and is based on the sequential enzymatic acylation—Mitsunobu inversion—enzymatic hydrolysis, which has been performed without isolation of the intermediates. In this way a 70–75% yield of 3-*O*-benzyl-*sn*-glycerol with 94–96% ee has been obtained.

1. Introduction

In the course of a project directed to the development of an efficient synthesis of phospholipids amenable of scale-up, we needed to elaborate a cost-effective enantioselective preparation of 1,2-*O*-*sn*-diacylglycerols (DAG) **1** (RCO_2H = fatty acid) (Scheme 1).



Scheme 1.

DAGs have been previously synthesised mainly starting from 3-*O*-benzyl-*sn*-glycerol **2**, that is in turn obtained in two steps from (*S*)-solketal **3**.[1] The typical synthesis of **3**, which is also commercially available, starts from D-mannitol. However, we wanted to use a more eco-friendly chemoenzymatic route that avoids the use of reagents such as $NaIO_4$ or $NaBH_4$. Various research groups have described chemoenzymatic approaches to **2** or **3**. Kinetic resolution has been accomplished by acylation of racemic **3** with various enzymes.[2–6] The enantioselectivity is not very high. In order to increase the ee, crystallisation methodologies[5] or double kinetic resolutions[4] have been devised. Lowering the temperature helps in increasing the ee, but larger amounts of enzyme are needed.[6] Also other cyclic derivatives of glycerol such as carbonates[7] have been resolved in this way. However, the main drawback of this approach is that the yields are lower than 40% and that the unwanted enantiomer has to be discarded.

This drawback is not present in other chemoenzymatic strategies, such as the asymmetrisation of 2-protected glycerols **4** to give chiral derivatives **5**[5] or the microbiological reduction of an asymmetrically diprotected dihydroxyacetone to give **6**.[8] In the first case, however, conversion of the chiral adduct **5** into **2** requires several synthetic steps, whereas in the second case the preparation of the starting ketone is rather long.

Vänttinen and Kanerva have demonstrated the possibility to overcome the main disadvantage of hydrolase catalysed kinetic resolutions, that is the loss of at least 50% of starting material, by coupling the enzymatic reaction with a Mitsunobu esterification.[3] We decided to use this method (that was never employed before on glycerol derivatives) for the enantioconvergent synthesis of

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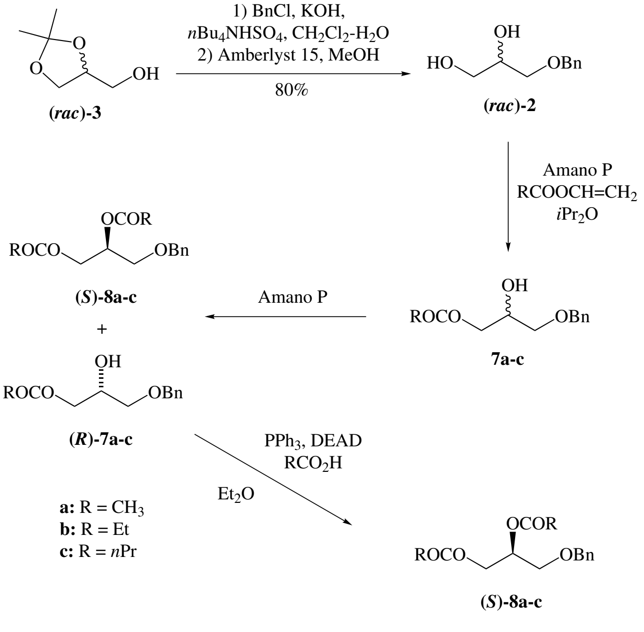
(*R*)-**2** and, in this paper, we report the achievement of this goal.

2. Results and discussion

Racemic solketal **3** was benzylated by a modification of the procedure of Hirt and Barner.[9] This method, that employs phase-transfer catalysis and benzyl chloride as the electrophile, is superior from a practical point of view than the most widely used reaction with NaH and benzyl bromide. We preferred *n*-Bu_4NHSO_4 as the catalyst, again for economical reasons. The intermediate *O*-benzyl solketal may be distilled as well, but its isolation is not necessary, and the following step can be performed directly on the crude product. Deblocking of the cyclic acetal was conveniently performed by the use of a resin bound acid catalyst (Amberlyst 15). In this way the work-up is very easy. Racemic **2** was purified by distillation.

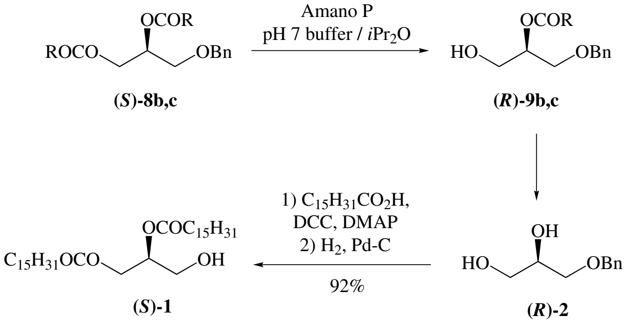
Resolution of racemic **2** through PFL (Fluka) or Amano P catalysed acetylation was already reported by Valverde and co-workers.[10] They demonstrated that acylation of the primary hydroxy group is much faster than that of the secondary one. While the first acylation proceeded with very low enantioselection, the second one afforded good levels of selectivity.

Due to the high cost of PFL, we repeated these experiments using Amano P lipase[11] (from *Pseudomonas cepacia*) (Scheme 2). The reactions were carried out starting with racemic **2** in THF–hexane 1:1, employing vinyl acetate as acyl donor. We did not isolate the intermediate monoacetates, but let the reaction proceed until the monoacetate **7** and the diacetate **8** were nearly equimolar. Upon chromatography we obtained (*S*)-**8a** with 86% ee, and (*R*)-**7a** with 80% ee. The **7**:**8** ratio was 48:52 (by NMR of the crude product).



Scheme 2.

Although it is not possible to calculate precisely the enantioselectivity factor E^{12} for the second acylation reaction, we can compute a 'virtual' *E*, from these two ee values. In the hypothesis that the first acylation is fully non-stereoselective, and that it is complete before the second one begins, the virtual value calculated in this way and the 'real' one will be coincident. These assumptions are reasonable, since we have seen that the first acylation affords regioselectively a nearly racemic monoacetate. Moreover only small quantities (1–2%) of the monoacetate **9** (Scheme 3), acylated at the secondary alcohol, may be detected by TLC and NMR during the course of the reaction. These 'virtual' *E* value may be useful because they give an idea of the overall selectivity of the two acylation steps. They can also be used for predicting the ees of **7** and **8** in function of the conversion. Actually, using the appropriate equations,[12] either from the two ee values or from one of them and the measured 48% conversion of the second acylation, we determined in all cases an *E* of about 33. This result was in line with what reported by Valverde and coworkers.[10]



Scheme 3.

Isolated (*R*)-**7a** (ee 80%) was then submitted to a Mitsunobu reaction with acetic acid in Et_2O. The reaction turned out to be quite slow. By stopping it after one day, only 65% conversion was measured. The ee of the product (*S*)-**8a** was 75%, indicating a nearly complete inversion. However, when the reaction was brought to completion, by carrying it out for prolonged time and through further additions of reagents, the ee of product (*S*)-**8a** was only 44%, indicating that the reaction was not stereoselective, probably because of intramolecular transacylation processes.

These unsatisfactory results prompted us to try different acylating agents, namely vinyl propionate and vinyl butyrate. The enzymatic acylations were carried out in diisopropyl ether, which, after a preliminary screening, was found to be the best solvent. From these reactions we could determine enantioselectivity factors of 23 and 35, respectively, for the propanoylation and the butyrylation.

The isolated monoesters (*R*)-**7b–c** were then submitted to the Mitsunobu esterification. In these two cases, the reactions in Et_2O were found to be completely stereospecific (inversion), also after long reaction times. Addition of DMF as cosolvent had an accelerating effect, but a slight lowering of the ees was observed. The reagents are in part consumed by a concurrent reaction that forms diethyl N,N-diacyl hydrazinodicarboxylate; the best procedure involves two or three further additions of the reagents (see Section 3) in order to drive the reaction to completion.

The Mitsunobu reaction was then repeated on the crude products coming from the enzymatic acylations. From the values of *E*, it is possible to calculate the ideal conversion of the second enzymatic acylation in order to get the best ee after the Mitsunobu step, in the hypothesis that the latter is quantitative and proceeds with complete stereospecificity.[3] In our case the optimum conversion should be 54% (with overall expected ees after Mitsunobu = 83% for **8b** and 87% for **8c**). However, the reactions tend to become very sluggish when approaching 50% conversion. This is clearly an advantage since a careful monitoring of the reaction is not necessary, but it is difficult to reach 54% conversion. The two reactions were therefore stopped at about 47% and the crude products submitted to the Mitsunobu conditions, giving, after chromatography, **8b** (77% yield, 82% ee) and **8c** (75% yield, 83% ee). The ees are even slightly higher than calculated, probably because the substitution reaction was not quantitative.

Although this two step methodology was demonstrated to be quite efficient, the ees of **8b** and **8c** were not yet high enough. Since for the obtainment of (*R*)-**2** we had to remove in some way the two acyl groups, we reasoned that, by performing this hydrolysis enzymatically,[13] we could further improve the ee. This 'double kinetic resolution' strategy had been used often in the past for increasing the optical purity.[4] We first explored the selectivity of the hydrolysis by using racemic **8b** and **8c**. Also in this case we did not isolate the intermediate monoesters **9**, but allowed the reaction to proceed until the ratio of **9** to **2** was close to 50%. In this case the reaction did not slow down very much at this point. The 'virtual' enantioselectivity factors were determined as for the acylation reactions and turned out to be in this case considerably lower (2.8 starting from **8b** and 5.7 starting from **8c**). The hydrolysis reactions were also less regioselective and we detected significant amounts of the regioisomeric monoacetates **7**. Notwithstanding these low *E* values, we calculated that, by carrying out the hydrolysis of enriched **8b** and **8c**, a high ee could be obtained by stopping the reaction at a **9**:**2** ratio of about 1:9.

This was indeed the case. When we submitted pure **8b** (82% ee) and **8c** (83% ee) to the enzymatic hydrolysis and stopped the reactions when the ratio **9**:**2** was equal to 11:89, we recovered (*R*)-**2** with 94% and 96% ee, respectively. The remaining **9b** and **9c** were also isolated and were demonstrated to have the opposite absolute configuration and ees = 36% and 40%, respectively. Thus double kinetic resolution is quite helpful in increasing the ees.

Having all the conditions set up, we performed the whole sequence without purification of the intermediates. As described in the experimental part, we only separated the diesters **8b** and **8c** from most of Ph_3PO and $EtO_2CNHNHCO_2Et$ by filtration after the Mitsunobu reaction. In the case of propanoylation, we obtained, with an excellent 75% overall yield, (*R*)-**2** with 94% ee. In the case of butyrylation, the overall yield was slightly lower (70%) and the ee was 96%.

While both procedures are valid, probably the one involving the dipropionate is better suited for a bulk synthesis, since vinyl propionate is cheaper than vinyl butyrate and because of the unpleasant odour of butyric acid. Although the enantioselectivity is slightly lower, a similar level of ee may be easily reached by stopping the final hydrolysis at a lower degree of conversion.

Finally we converted this intermediate into 1,2-dipalmitoyl-*sn*-glycerol (*S*)-**1**, following essentially the method previously described by Martin.[14] The overall yield of this DAG from racemic solketal was 55%. The synthetic sequence involved reactions taking place at 0–50 °C, without the need of inert atmosphere, and with low cost and easily handled reagents. The intermediates either did not require purification or were conveniently purified by distillation or crystallisation. Therefore we think that this enantioselective synthesis of DAG may be conveniently up-scaled for an industrial production. This will make biologically important phospholipids more easily accessible.

3. Experimental

3.1. (*rac*)-1-*O*-Benzylglycerol 2

A solution of *rac* 2,2-dimethyl-1,3-dioxolane-4-methanol (10 mL, 80.4 mmol) in CH_2Cl_2 (50 mL) was treated sequentially with 30% (w/v) aqueous NaOH (37.8 mL, 283.5 mmol), *n*-Bu_4NHSO_4 (2.730 g, 8.04 mmol) and benzyl chloride (10.18 mL, 88.5 mmol). The mixture was vigorously stirred at 50 °C for 25 h. After cooling, it was diluted with saturated aqueous NaCl and extracted with Et_2O. After washing the organic extracts with saturated NaCl, and drying (Na_2SO_4), evaporation gave a crude oil. It was taken up in MeOH (50 mL) and treated with 4.0 g of Amberlyst 15. After stirring for 5 h at rt, the suspension was filtered and the resin was washed with AcOEt/MeOH 1:1. $CaCO_3$ (1.0 g) was added to the filtrate and, after stirring for 10 min, the mixture was filtered again. Evaporation and distillation (128–130 °C at 0.15–0.2 mbar) gave pure (*rac*)-**2** (11.74 g, 80%).

3.2. 3-*O*-Benzyl-*sn*-glycerol (*R*)-2

A solution of (*rac*)-**2** (10.38 g, 57.0 mmol) in diisopropyl ether (155 mL) was treated with vinyl propionate (31 mL, 285 mmol) and with Amano P lipase (5.43 g). After stirring at 20 °C for 66 h, the suspension was filtered and evaporated to dryness. The ratio monopropionate **7b**:dipropionate **8b** was 53:47 (^1H NMR). The crude oil was taken up in dry Et_2O (150 mL) and treated with triphenylphosphine (5.07 g, 19.3 mmol), diethyl azodicarboxylate (DEAD) (3.0 mL, 19.05 mmol) and propionic acid (1.43 mL, 19.2 mmol). After 17 h a further aliquot of PPh_3 (2.157 g, 8.2 mmol), DEAD (1.30 mL, 8.26 mmol) and propionic acid (0.61 mL, 8.18 mmol) was added. This operation was repeated after 22 h and 37 h. After further stirring for 18 h (the complete disappearance of monopropionate was detected by TLC), the mixture was filtered, washed with saturated aqueous $NaHCO_3$ and evaporated to dryness. It was taken up in petroleum ether/Et_2O 2:1 (50 mL), filtered through a sintered funnel and evaporated to dryness. The crude product was taken up in *i*-Pr_2O (80 mL), and treated with 1 M pH 7 phosphate buffer (KH_2PO_4–K_2HPO_4) (240 mL). Amano P lipase (8.2 g) was added. The mixture was stirred at 20 °C for 34 h. The mixture was saturated with NaCl and filtered through a Celite cake. Extraction with AcOEt gave, after evaporation, a crude product. ^1H NMR indicated a ratio monopropionate **9b**:diol **2** = 11:89. The crude product was chromatographed on silica gel (CH_2Cl_2 to CH_2Cl_2/MeOH 97:3) to give pure (*R*)-**2** (7.78 g, 75%). Alternatively it can also be purified by distillation. This product had an ee of 94%. The spectroscopic and polarimetric data were in accord with those reported in the literature. The absolute configuration was also confirmed by transformation into the diacetyl derivative (*S*)-**8a** (Ac_2O, pyridine), which had $[\alpha]_D = +16.3$ (*c* 1.64, $CHCl_3$). Lit.[10] = +14.0 (*c* 0.5, $CHCl_3$).

By a similar procedure, (*R*)-**2** was also obtained through the dibutyrate in 71% overall yield and with an ee of 96%.

3.3. Determination of enantiomeric excesses of 7a–c, 8a–c, 2

Compounds **7a–c** and **8a–c** were all hydrolysed with 0.35 M KOH in MeOH–H_2O 3:1 to the diol **2**. This was in turn converted into the double Mosher ester, by reaction with (*R*) α-methoxy-α-phenyl-α-(trifluoromethyl)acetyl chloride and 4-dimethylaminopyridine in CH_2Cl_2. The enantiomeric excess was then determined by ^1H NMR or by HPLC (Daicel Chiralpak AD, *n*-hexane–*i*-PrOH 9:1, flow: 0.5 mL/min, detection: UV-DAD (220, 230, 250 nm)).

3.4. 3-*O*-Benzyl-1,2-*O*-dipalmitoyl-*sn*-glycerol (*S*)-1

A solution of (*R*)-**2** (7.00 g, 38.4 mmol) in CH_2Cl_2 (150 mL) was treated with palmitic acid (22.64 g, 88.3 mmol), and 4-dimethylaminopyridine (938 mg, 7.68 mmol), cooled in an ice bath, and treated with a solution of dicyclohexylcarbodiimide (18.22 g, 88.3 mmol) in CH_2Cl_2 (100 mL). The resulting suspension was stirred at 0 °C for 1 h and at rt overnight. After dilution with 100 mL of petroleum ether, the dicyclohexylurea was removed by filtration. The filtrate was washed with 5% $NaHCO_3$, with 1 M HCl, and with saturated NaCl to give, after evaporation, a solid that was triturated from MeOH (24.81 g, 96%). Mp: 64–65 °C.

This solid was taken up in 300 mL of 96% EtOH and 30 mL of glacial acetic acid. The solution was hydrogenated at room temperature on 10% Pd–C (500 mg) at atmospheric pressure. As soon as the reaction was complete, the catalyst was removed by filtration on Celite pad. Evaporation afforded a solid that was recrystallised from *n*-hexane to give 19.98 g of **1** (93%). Mp: 62.8–64.6 °C. The spectroscopic and polarimetric data were in accordance to those reported in the literature.[14,15]

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References

1. Jurczak, J.; Pikul, S.; Bauer, T. *Tetrahedron* **1986**, *42*, 447–488.
2. Partali, V.; Melbye, A. G.; Alvik, T.; Anthonsen, T. *Tetrahedron: Asymmetry* **1992**, *32*, 65–72; Jungen, M.; Gais, H.-J. *Tetrahedron: Asymmetry* **1999**, *10*, 3747–3758; Vänttinen, E.; Kanerva, L. T. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3459–3463; Vänttinen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1996**, *7*, 3037–3046; Wang Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205; Ladner, W. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7250–7251; Parmar, V.; Prasad, A. K.; Singh, P. K.; Gupta, S. *Tetrahedron: Asymmetry* **1992**, *3*, 1395–1398.
3. Vänttinen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1995**, *6*, 1779–1786.
4. Vänttinen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1997**, *8*, 923–933.
5. Bianchi, D.; Bosetti, A.; Golini, P.; Cesti, P.; Pina, C. *Tetrahedron: Asymmetry* **1997**, *8*, 817–819.
6. Sakai, T.; Kishimoto, T.; Tanaka, Y.; Ema, T.; Utaka, M. *Tetrahedron Lett.* **1998**, *39*, 7881–7884.
7. Pallavicini, M.; Valoti, E.; Villa, L.; Piccolo, O. *J. Org. Chem.* **1994**, *59*, 1751–1754; Hamaguchi, S.; Yamamura, H.; Hasegawa, J.; Watanabe, K. *Agric. Biol. Chem.* **1985**, *49*, 1509–1511.
8. Aragozzini, F.; Maconi, E.; Potenza, D.; Scolastico, C. *Synthesis* **1989**, 225–227.
9. Hirt, G.; Barner, R. *Helv. Chim. Acta* **1982**, *65*, 1059–1084.
10. Herradon, B.; Cueto, S.; Morcuende, A.; Valverde, S. *Tetrahedron: Asymmetry* **1993**, *4*, 845–864.
11. We wish to thank Amano for a kind gift of this enzyme.
12. Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*; Wiley-VCH: Weinheim, 1999.
13. Poppe, L.; Novák, L.; Kajtár-Peredy, M.; Szántay, C. *Tetrahedron: Asymmetry* **1993**, *4*, 2211–2217.
14. Martin, S. F.; Josey, J. A.; Wong, Y.-L.; Dean, D. W. *J. Org. Chem.* **1994**, *59*, 4805–4820.
15. Müller, H. K.; Burgold, J. *Tetrahedron Lett.* **1986**, *42*, 1533–1538; Reddy, K. K.; Saady, M.; Falck, J. R.; Withed, G. *J. Org. Chem.* **1995**, *60*, 3385–3390; Bruzik, K. S.; Salamonczyk, G.; Stec, W. J. *J. Org. Chem.* **1986**, *51*, 2368–2370.