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(54) **Enantio- and regioselective syntheses of organic compounds using enol esters as irreversible transacylation reagents**

Enantio- und regioselektive Synthesen von organischen Verbindungen mit Enolestern als irreversibele Transacylierungs-Reagenzien

Synthèses enantio- et régioselectives de composés organiques avec des esters énoliques comme réactifs irréversibles de transacylation

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- **TETRAHEDRON LETTERS. vol. 28, no. 9, 1987, OXFORD GB pages 953 - 954 M.DEGUEIL ET AL. 'Enzymatic reactions in organic synthesis:2-Ester interchange of vinyl esters.'**
- **JOURNAL OF ORGANIC CHEMISTRY. vol. 53, no. 13, 24 June 1988, EASTON US pages 3127 - 3129 YI-FONG WANG ET AL. 'Lipase-catalyzed irreversible transesterification for preparative synthesis of chiral glycerol derivatives'**
- **JOURNAL OF THE AMERICAN CHEMICAL SOCIETY. vol. 110, 1988, GASTON, PA US pages 7200 - 7205 YI-FONG WANG ET AL. 'Lipase-catalyzed irreversible transesterification using enol esters as acylating reagents :Preparative enantio- and regioselectve syntheses of alcohols, glycerol derivatives, sugars and organometallics.'**
- **PATENT ABSTRACTS OF JAPAN vol. 13, no. 447 (C-642)(3795) 6 October 1989**

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- AGRICULTURAL AND BIOLOGICAL CHEMISTRY. vol. 53, no. 7, July 1989, TOKYO JP pages 1879 - 1884 M.INAGAKI ET AL. 'Lipase-catalyzed stereoselective acylation of (1,1¡-binaphtyl)-2,2¡-diol and deacylation of its esters in an organic solvent.'
- JOURNAL OF ORGANIC CHEMISTRY. vol. 53, no. 26, 23 December 1988, EASTON US pages 6130 - 6133 J.HIRATAKE ET AL. 'Irreversible and highly enantioselective acylation of 2-halo-1-arylethanols in organic solvents catalyzed by a lipase from pseudomonas fluorescens.'

Description

BACKGROUND OF THE INVENTION

[0001] The present invention relates to enantio- and regioselective synthesis of esters of alcohols, sugars, organometallics and glycosides and to their preparation using enzyme mediated transesterification. More particularly, the present invention relates to protease catalyzed irreversible transesterification using enol esters as transacylation reagents.

[0002] Hydrolytic enzymes such as lipases, esterases, and proteases have been used extensively as catalysts in enantioselective syntheses. Whitesides, G.M., Wong, C-H. Angew. Chem. Int. Ed. Engl. 24 (1985) 617; Jones, J.B. Tetrahedron 42 (1986) 3351; Roberts, S.M. Chem. Br. (1987) 127; Akiyama, A., Bednarski, M., Kim, M.J., Simon, E.S., Waldmann, H.I., Whitesides, G.M. Ibid. (1987) 645. Because of their relatively high stability in organic media, many hydrolytic enzymes also can be used in organic solvents for certain types of transformations which are difficult to do in water. The most common reactions are esterase and lipase-catalyzed stereoselective esterifications and transesterifications. Klibanov, A.M. CHEMTECH (1986) 354-9; Klibanov, A.M., Cambou, B. J. Am. Chem. Soc. 106 (1984) 2687-92. Chen, C-S., Wu, S-H., Girdaukas, G., Sih, C.J. J. Am. Chem. Soc. 109 (1987) 2812-17; Guo, Z.W., Sih, C.J. Ibid. 110 (1988) 1999-2001; Gil, G., Ferre, E., Meou, A., Petit, J.L., Triantaphylides, C. Tetrahedron Lett. 28 (1987) 1647; Yokozeki, K., Yamanaka, S., Takinami, K., Hirose, Y., Tanaka, A., Sonomoto, K., Fukui, S. Eur. J. Appl. Microbiol. Biotechnicol 14 (1982) 1; Tambo, G.M.R., Schar, H-P., Busquets, X.F., Ghisalba, O. Tetrahedron Lett. 27 (1986) 5705-10; Belan, A., Bolte, J., Fauve, A., Gourey, J.G., Veschambre, H. J. Org. Chem. 52, 256-60. Langrand, G., Baratti, J., Buono, G., Triantaphylides, C. Tetrahedron Lett. 27 (1986) 29-32.

[0003] The J. of Org. Chem., Vol. 53, No. 13 (June 1988) p. 3127 - 3129 describes the preparation of a stereoselectively acylated alcohol by mixing a meso-alcohol and an enol ester in the presence of a lipase.

[0004] One disadvantage of enzyme catalyzed hydrolytic reactions is that they are very slow compared to simple hydrolyses. Langrand, G., Baratti, J., Buono, G., Triantaphylides, C. Tetrahedron Lett. 27 (1986) 29-32. In addition, the products produced by enzymatic hydrolyses very often have to be separated from other by-products. (particularly alcohol generated from the acylating reagent). Due to the reversible nature of these reactions, and due to the same stereoselectivity of the enzyme catalysis in both directions, the optical purity of the product obtained decreases as the reverse reaction proceeds. This situation is illustrated in FIG. 1 where a racemic alcohol is to be resolved via an enzymatic esterification (R" = H) or transesterification.

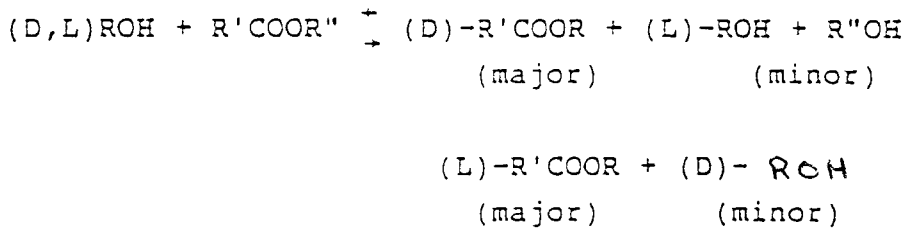


FIG. 1

[0005] As shown in FIG. 1, if the D-isomer is a better substrate than the L-isomer for the enzyme, accumulation of the D-ester and the unreactive L-alcohol will be observed. In the reverse reaction, however, the D-ester is a better substrate and will be converted to the D-alcohol. The enantiomeric excess of both the D-ester and the L-alcohol therefore will decrease progressively as the extent of the reverse reaction increases. This reverse reaction problem clearly has been illustrated in the kinetic resolution of menthol, Chen, C-S., Wu, S-H., Girdaukas, G., Sih, C.J. J. Am. Chem. Soc. 109 (1987) 2812-17; Guo, Z.W., Sih, C.J. Ibid. 110 (1988) 1999-2001, and can be seen in the enantioselective esterification or transesterification of meso compounds.

SUMMARY OF THE INVENTION

[0006] The method of the present invention blocks the progress of the reverse reaction. The present invention is a process for irreversible regio- and stereoselective protease catalyzed acylation of alcohols using enol esters as acylating reagents. The present invention permits the selective modification of hydroxyl group(s) of chiral alcohols, including

sugars, organometallics and glycosides. The enol freed upon transesterification rapidly tautomerizes to the corresponding volatile aldehyde or ketone thereby preventing the reverse reaction from occurring.

DETAILED DESCRIPTION OF THE INVENTION

[0007] Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-200E spectrometer. All chemical shifts were reported in ppm using tetramethylsilane as an internal standard unless otherwise indicated. Rotations were determined on a Perkin Elmer 240 polarimeter. Gas chromatographic (GC) analyses were performed on a Hewlett-Packard 5890 instrument with a 20-m DB-5 megabore column.

[0008] Vinyl acetate ($5/Kg, bp 72°C) and isopropenyl acetate ($25/Kg, bp 94°C) were from Aldrich Chemical Co. Vinyl propionate ($25/25 g, bp 93-94°C) was from Pfaltz and Bauer, Inc. Some experimental protocols are described in Tables 1, 3 and 4.

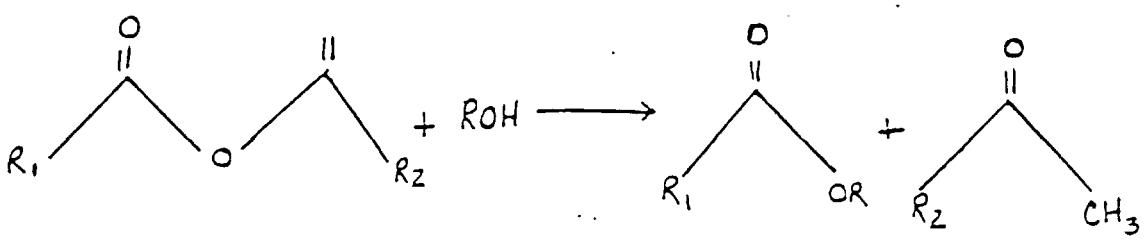
[0009] The procedure for preparation of isopropenyl valerate (1b of FIG. 2) was similar to that reported for the preparation of other isopropenyl esters, with some modifications. Rothman, E.S., Serota, S., Perlstein, T., Swern, D. J. Org. Chem. 27 (1962) 3123-27. To a 250 mL round bottom flask was added 10 mL of valeric acid (91.9 mmol) which had been freshly distilled, and 20mL of valeric anhydride. Then, 200 mL of freshly distilled isopropenyl acetate was added followed by 2 drops of concentrated sulfuric acid. The mixture then was heated to reflux under an atmosphere of argon for 10 h, after which time all of the valeric acid had been consumed as evidenced by capillary GC. The reaction mixture was allowed to cool to room temperature and 0.5 g of sodium bicarbonate was added to quench the acid catalyst. The isopropenyl acetate then was removed by evaporation under reduced pressure. The orange liquid remaining was poured into 300 mL of 0°C saturated sodium bicarbonate which was overlayed with 100 mL of diethyl ether. The mixture was stirred vigorously and the ether layer was analyzed by GC for the disappearance of the mixed valeric acetic anhydride. After all of the anhydride was consumed (6 h) the ether layer was separated and the aqueous layer was washed with 100 mL of ether. The combined ether layers were washed with 5 x 25 mL portions of saturated sodium bicarbonate to remove the valeric acid. The ether layer then was washed with saturated brine (30 mL) and the ether was then dried over sodium sulfate. The ether was removed under reduced pressure and the isopropenyl ester was purified by vacuum distillation (bp = 50-52°C, 8 mm Hg). 7.85 g of a clear colorless liquid (1b) was obtained (60.1% yield). ^{1}H-NMR ($CDCl_3$) 4.65 (m, 2H), 2.35 (t, 2H) 1.90 (s, 3H), 1.65 (m, 2H), 1.35 (m, 2H), 0.90 (s, 3H). ^{13}C-NMR 171.89, 153.00, 101.87, 34.02, 26.92, 22.16, 19.52, 13.16. In a similar manner isoprooenyl butyrate was prepared from butyric acid in 54% yield. 3.68 g of isopropenyl butyrate were prepared from 4.85 mL of butyric acid and 10 mL of butyric anhydride. ^{1}H-NMR 4.60 (m, 2H), 2.30 (t, 2H), 1.85 (s, 3H), 1.60 (m, 2H), 0.90 (t, 3H).

[0010] The method of Swern and Jordan was used to prepare vinyl valerate (1e of FIG. 2). Swern, D., Jordan, E.F. Organic Synthesis, Coll. Vol. IV (1963) 977-80, incorporated herein by reference. Freshly distilled valeric acid (40 mL, 0.37 mol) and vinyl acetate (300 mL) were placed in a 3-necked 500 mL round bottomed flask fitted with a reflux condenser, a gas inlet tube and a thermometer. The solution was stirred under argon and mercuric acetate (1.2 g, 0.37 mmol) was added. The reaction mixture was stirred under argon for 30 min, after which time 10 drops of 100% sulfuric acid was added. The solution was heated to reflux for 6 h and then was allowed to cool to room temperature. Sodium acetate (1.0 g) was added to quench the acid catalyst. The excess vinyl acetate was removed by distillation under argon. The product (vinyl valerate) 1e was isolated by distillation (bp = 135-145°C) as a clear colorless liquid (29.4 g, 62% yield). ^{1}H-NMR 7.24 (m, 1H), 4.80 (m, 1H), 4.48 (m, 1H), 2.32 (t, 2H), 1.60 (m, 2H), 1.30 (m, 2H), 0.85 (t, 3H). ^{13}C-NMR 170.69, 141.11, 97.22, 33.54, 26.57, 22.10, 13.57.

[0011] Any chiral alcohol having no excessive steric hindrance can be used in the present method. Structures 15 and 16 of Table 1 represent compounds wherein excessive steric hindrance is present.

Catalyzed Reactions

[0012] A number of catalyzed irreversible transesterifications using enol esters as acylating reagents can be performed in a manner outlined generally in FIG. 2.



	R_1 e.g.	$R2_2$ e.g.	
1a	CH_3-	CH_3	isopropenyl acetate
1b	$CH_3(CH_2)_3$-	CH_3	isopropenyl valerate
1c	CH_3-	H	vinyl acetate
1d	CH_3CH_2-	H	vinyl propionate
1e	$CH_3(CH_2)_3$-	H	vinyl valerate

FIG. 2

[0013] The reactions will result in optically active esters from several alcohols including those from glycerol and serinol derivatives, organometallics, nucleoside derivatives, sugars, and other chiral alcohols.

[0014] The alcohol substrate and an excess of an enol ester are dissolved in an organic solvent, such as pyridine or a less polar solvent. After a catalytic amount of enzyme was added, the suspension is incubated. The reaction can be monitored analytically for conversion. Once the required extent of conversion is reached, the desired product is isolated; e.g. the solvent removed by evaporation in a vacuum. The ester product and the unreacted alcohol can be separated by chromatography on a silica gel column.

[0015] Preferred acylating reagents (enol esters) are isopropenyl acetate, isopropenyl valerate, vinyl acetate, vinyl valerate or vinyl propionate, and among these especially vinylacetate, vinyl propionate or isopropenyl acetate.

[0016] Preferred substrates are 2-N-benzyloxycarbonyl (Z) serinol, glyceridol, solketal, (±)2-octanol, sulcatol or ferrocenyl ethanol.

[0017] Furthermore, the enzyme can advantageously be immobilized on a support. A preferred protease is protease N.

[0018] The reaction schemes for enantioselective acylation of 2-O-benzylglycerol (2) and N-carbobenzoxy serinol (5) are shown in FIGS. 2 and 4, respectively.

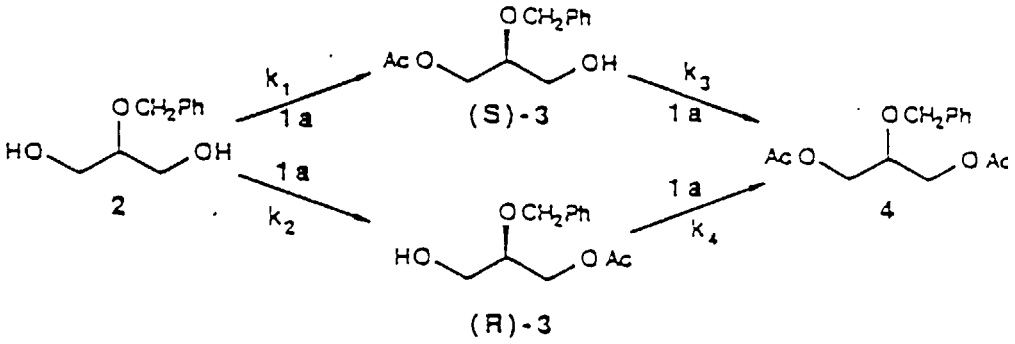


FIG. 3

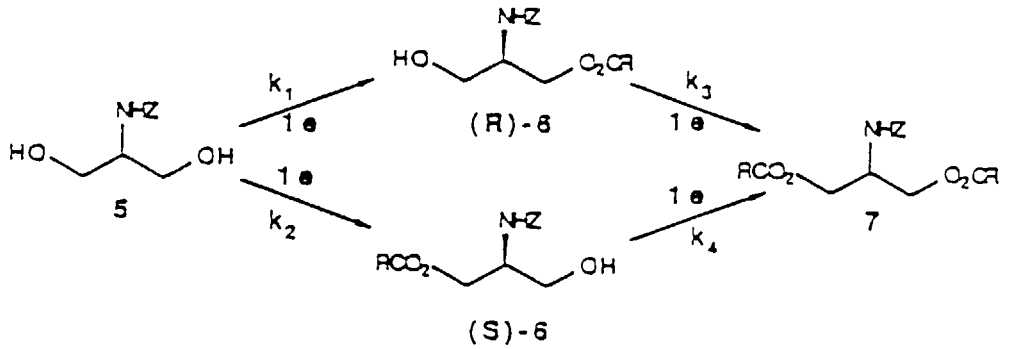
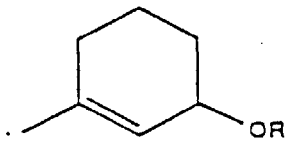


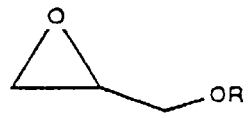
FIG. 4

[0019] Table 1 diagrams possible starting materials and products which can be achieved by catalyzed transesterification.

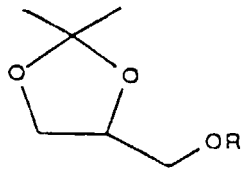
Table 1



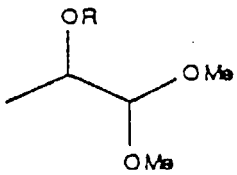
8a R = H
8b R = Ac



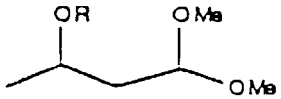
9a R = H
9b R = propionyl



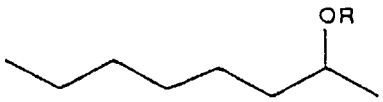
10a R = H
10b R = Ac
10c R = valeryl



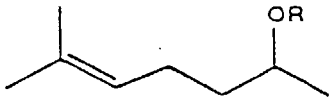
11a R = H
11b R = Ac
11c R = propionyl
11d R = valeryl



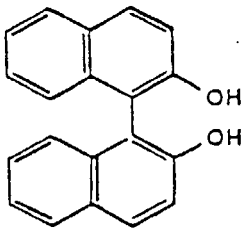
12a R = H
12b R = Ac
12c R = propionyl



13a R = H
13b R = Ac



14a R = H
14b R = Ac



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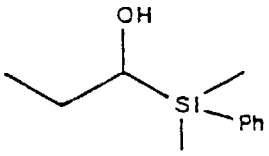

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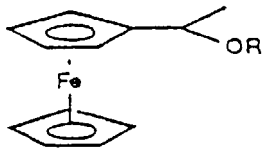


17a R = H
17b R = propionyl

[0020] In the following the general procedure using protease catalyzed transesterification is described:

[0021] To determine the constants (kinetic parameters), the dials, monoesters, and diesters were determined by GC analysis at a certain degree of conversion. The enantiomeric compositions of monoesters were determined by NMR analysis.

[0022] The kinetics of these irreversible transesterifications can be treated as similar to the kinetics Of hydrolysis, and the equation developed by Sih et al. for use in prediction of ee vs. conversion in hydrolysis should be applicable here. Wang, Y.F., Chen, C.S., Girdaukas, G., Sih, C.J. J. Am. Chem. Soc. 106 (1984) 3695; Wang, Y.F., Sih, C.J. Tetrahedron Lett. 25 (1985) 4999. To determine the constants, the diols, monoesters, and diesters were determined by GC analysis at certain degree of conversion. The enantiomeric compositions of monoesters were determined by NMR analysis.

Structural effect and solvent of enol esters

[0023] The structure of the enol ester can vary depending on the rate and stereoselectivity. The same applies to the solvent applied. Suitable esters are CH_3CO_2Et, $CH_3CO_2CH_2CF_3$, CH_3CO_2ET, the aforementioned structures 1a - 1e. Suitable solvents are benzene, isopropenyl acetate, chloroform, THF (Tetrahydrofuran), DMF, pyridine etc.

[0024] Moreover, the combination of two irreversible enzymatic processes, ester hydrolysis and ester synthesis, enabled effective syntheses of a number of optically active monoesters and acohols in both enantiomeric forms even with a moderately enantioselective enzyme.

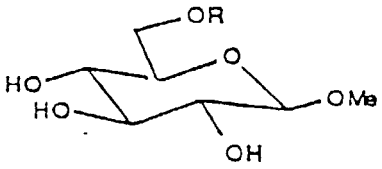
[0025] The leaving groups (acetone and acetaldehyde) of enol esters used in the processes are volatile and easy to remove, making the product separation very simple. When the transesterification reaction is carried out in neutral apolar organic solvents, the procedure is suitable for acid-, base- or water sensitive substances.

Regioselective Acylations of Sugars and Their Derivatives

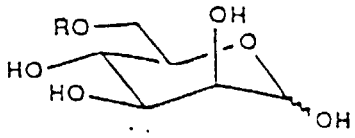
[0026] The methyl and higher glycosides of hexoses and pentoses are sufficiently soluble in pyridine or other less polar media such that the enzymatic acetylations of these compounds can be accomplished with lipase-catalysis. Stronger solvents such as N,N-dimethylformamide (DMF), dissolve many otherwise insoluble sugars but they also render the lipases inactive. Riva, S., Chapineau, J., Kieboom, A.P.G., Klibanov, A.M. J. Am. Chem. Soc. 110 (1988) 584-589. It presently has now surprisingly been found that Protease N (neutral protease from Amamo International Enzyme Company) will utilize enol esters as acyl donors. This enzyme also retains its catalytic activity in dry DMF, contrary to lipase reactions.

[0027] Summaries of some of the data obtained with hexoses (Table 2), and nucleosides (Table 3) are shown hereinafter. Selected specific as well as general procedures for acetylation of sugars and their derivatives also are disclosed.

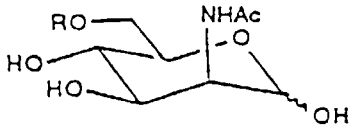
Table 2. Enzyme-catalyzed acetylation of hexoses and their derivatives using enol esters.



18a R = H
18b R = Ac



19a R = H
19b R = Ac



20a R = H
20b R = Ac

Compound	ENZ	Enol ester	Conversion (%)	Regioselectivity (%)	Isolated Yield (%)
18a	CCL*	1c	30	>98	23
19a	PN	1a	60	>90	49
20a	PN	1a	85	>90	73

* Candida cylindracea, Tuppe VIII, Sigma Chemical Company; Comparison example (example 1)

Example 1 - CCL-catalyzed transesterification of methyl β-D-glucopyranoside (18a of Table 2) with vinyl acetate (1c of FIG. 2)(comparison)

[0028] Methyl β-D-glucopyranoside (18a of Table 2) (388 mg, 2 mmol) and vinyl acetate (1c of FIG. 2) (4 mmol) were dissolved in 12 mL of benzene - pyridine (2:1). Then 388 mg of CCL was added, and the suspension was stirred at 28°C. After 24 hours, an additional 388 mg of CCL was added, and this was repeated after 48 hours. The suspension was stirred at 28°C for 5 days; then worked up as usual to afford methyl 6-O-β-D-glucopyranoside 18b as a solid, which was crystallized from ethyl acetate-n-hexane; m.p. 129 ≃ 130°C; $[\alpha]^{25}_D$ -27.1 (c 1.4, CH_3OH); ^{1}H-NMR (CD_3COCD_3); 2.02 (3H, s); 2.98 (1H, s); 3.13 ≃ 3.25 (1H, m), 3.3 ≃ 3.55 (3H, m), 3.45 (3H, s), 4.15 ≃ 4.25 (2H, m); 4.30 ≃ 4.45 (3H, m); ^{13}C-NMR (CD_3COCD_3); 104.56 (Cl), 74.29 (C2), 77.36 (C3), 70.85 (C4), 74.33 (C5), 64.01 (C6), 20.42 and 170.69; (acetyl), 56.39 (methoxy).

[0029] Protease N obtained from Amano International Enzyme Co. was used on the following reactions. Other highly stable proteases, such as proteases obtained from thermophillic organisms or genetically engineered stable proteases, also could be used in the following reactions. The crude commerical preparation was dissolved in 0.1 M phosphate buffer, pH 7.8 (2 g/35 mL) and lyophilized. The dry powder that was obtained was pulverized with a mortar and pestle prior to use.

Regioselective Acylation of Sugar

Example 2 - Preparation of 2-acetamido-6-O-acetyl-2-deoxy-D-mannopyranose

[0030] Protease N from Bacillus subtilis (obtained from Amano) (2 g) was dissolved in 0.1 mol NaH_2PO_4 (35 mL), and the resulting solution was stirred for 15 min. The pH was then adjusted to 7.8 with 8.0 NaOH and the solution was freeze-dried. This freeze-dried preparation was used in the synthetic procedure. N-acetyl-β-D-mannosamine monohydrate (Sigma) (478 mg, 2 mmol) was suspended in anhydrous N N-dimethylformamide (2 mL). Isopropenyl acetate (600 mg, 6 mmol) was added followed by the enzyme preparation (600 mg). The suspension was shaken at 45°C and monitored by TLC (silica gel; EtOAc:MeOH:H_2O = 100:10:1). After 44 h, the suspension was filtered and the enzyme washed with methanol (2 x 3 mL). The solvents were evaporated under vacuum at 40°C to give a yellow syrup. This syrup was fractionated on a silica gel column (45 g) eluted with EtOAc/MeOH/H_2O = 100/10/1. Two products were obtained: the first with a higher R_f corresponded to a triacetate compound (30 mg, 10%). The second (major) product was obtained as an amorphous white solid which, upon analysis was revealed to be 2-acetamido-6-O-acetyl-2-dexoy-D-mannopyranose. (384 mg, 73%): ^{1}H-NMR (D_2O/p-dioxane = 3.57 ppm) δ 4.93 (s, H 1 α), 4.84 (s, H 1 β), 4.29-4.02 (m, 5 H), 3.87 (dd, H 3 α), 3.70-3.27 (m, 3 H), 1.95, 1.91, 1.88, and 1.87 (4 s, 6 H, acetal); ^{13}C-NMR (D_2O/p-dioxane = 67.46 ppm) δ 176.60, 175.67, 174.96, and 174.92 (all carbonyls), 94.05 (c 1 β), 93.97 (C 1 α), 74.78 (c β), 72.72 (C β), 70.57 (C 5 α), 69.43 (C $^{\alpha}$), 67.94 (C α), 67.78 (C β), 64.61 (C 6 α), 64.36 (C 6 β), 54.84 (C 2 β), 54.18 (c 2 α); α/β = 76/24; mp 47 - 51°C; $[\alpha]^{24}_D$ +15.9° (c 1.13 H_2O). Anal. Calcd for $C_{10}H_{16}NO_7$: C, 45.80; H, 6.15; N, 5.34. Found: C, 45.89; H, 6.20; N, 4.95.

Regioselective Acylation of Nucleosides

Example 3 - General Procedures

[0031] The following general procedures were used in performing the regioselective acylation of nucleosides listed in Table 3:

[0032] 1 mmol of nucleoside was dissolved in 2-4 mL of dry DMF and warmed. The solution was cooled to 45°C and 1.1 mL (10 eq) of isopropenyl acetate and 260 mg of pulverized protease N were added. The suspension was shaken at 45°C. After the appropriate times, as indicating in Table 7, the reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was purified by silica gel chromatography using mixtures of ethyl acetate:ethanol:water as the eluent for the times indicated. The isolated products were obtained in the yields shown in Table 3.

[0033] Table 3 indicates that, where acetylation occurred, the monoacetyl derivative was predominately formed. The preferential formation of the monoacetyl derivative indicates that the nucleoside was acetylated at the primary (5') hydroxyl group.

Table 3

Selective Enzymatic Acetylations of Nucleosides and Sugars in Anhydrous Dimethyl-Formamide					
Substrate	Enzyme	Time (days)	Monoacetyl (%)	Diacetyl (%)	Starting Material (%)
Guanosine	PN	5	0	0	100
Adenosine	PN	1.75	40	0	60
"	PN	5	65	<5	30
2-Deoxy adenosine	PN	2	50	-	-
"	PN	4	80	-	-
Uridine	PN	1.75	50	0	50
"	PN	5	80	<5	15
"	PN(pyr)	5	60	0	40
"	PN(THF)	5	0	0	100
Cytidine	PN	1.5	60	0	40
"	PN	3	80	<5	15
"	S	3	0	0	100
2-Deoxycytidine	PN	2	60	-	-
"	PN	4	80	-	-
Thymidine	PN	1.5	90	0	10
"	S	1.5	0	0	100
Methyl 2-deoxy-D-ribofuranoside	PN	2	70	-	-
PN = protease N [Amano] S = subtilisin BPN					

[0034] The simplicity of this irreversible transesterification makes the operation useful for the preparation of chiral alcohols or esters that may be difficult to prepare by other means.

Claims

1. A method for preparing a stereoselectively acylated alcohol, comprising

   mixing a chiral alcohol substrate and an excess of enol ester in an organic solvent of similar polarity to the alcohol substrate to form a mixture;

   incubating said mixture with a catalytic amount of a protease, and

   isolating from said mixture an optically enriched stereoselectively acylated form of said alcohol substrate.

2. The method of claim 1 wherein the acylating reagent is isopropenyl acetate, isopropenyl valerate, vinyl acetate, vinyl valerate or vinyl propionate.

3. The method of Claim 1 wherein said enzyme is immobilized on a support.

4. The method of Claim 1 wherein said substrate is 2-N-benzyloxycarbonyl (Z) serinol, glyceridol, solketal, (±)2-octanol, sulcatol or ferrocenyl ethanol.

5. The method of Claim 1 wherein said protease is immobilized on a support.

6. The method of Claim 4 wherein the acylating reagent is vinyl acetate or vinyl propionate.

7. The method of Claim 1 wherein the acylating reagent is vinyl acetate or isopropenyl acetate.

8. The method of any of claims 1 - 7 wherein the protease is protease N.

Patentansprüche

1. Ein Verfahren zur Herstellung eines stereoselektiv acylierten Alkohols, umfassend:

   Vermischen eines chiralen Alkoholsubstrats und eines Überschusses an Enolester in einem organischen Lösungsmittel von gleicher Polarität zum Alkoholsubstrat unter Bildung eines Gemischs;

   Inkubieren dieses Gemischs mit einer katalytischen Menge einer Protease und

   Isolieren einer optisch angereicherten stereoselektiv acylierten Form dieses Alkohoisubstrats von diesem Gemisch.

2. Das Verfahren des Anspruchs 1, worin das Acylierungsreagenz Isopropenylacetat, Isopropenylvalerat, Vinylacetat, Vinylvalerat oder Vinylpropionat ist.

3. Das Verfahren von Anspruch 1, worin dieses Enzym immobilisiert ist auf einem Träger.

4. Das Verfahren von Anspruch 1, worin dieses Substrat 2-N-Benzyloxycarbonyl (Z) Serinol, Glyceridol, Solketal, (±)2-Octanol, Sulcatol oder Ferrocenylethanol ist.

5. Das Verfahren des Anspruches 1, worin diese Protease auf einem Träger immobilisiert ist.

6. Das Verfahren des Anspruchs 4, worin das Acylierungsmittel Vinylacetat oder Vinylpropionat ist.

7. Das Verfahren des Anspruchs 1, worin das Acylierungsmittel Vinylacetat oder Isopropenylacetat ist.

8. Das Verfahren nach einem der Ansprüche 1 - 7, worin die Protease Protease N ist.

Revendications

1. Méthode de préparation stéréosélective d'un alcool acylé comprenant

   le mélange d'un substrat constitué par un alcool chiral et d'un ester d'énol en excès dans un solvant organique ayant une polarité similaire au substrat alcoolique pour former un mélange ;
   la mise en incubation dudit mélange avec une quantité catalytique d'une protéase et
   l'isolement dudit mélange d'une forme acylée dudit substrat alcoolique enrichie optiquement de façon stéréosélective.

2. Méthode selon la revendication 1, dans laquelle le réactif acylant est l'acétate d'isopropényle, le valérate d'isopropényle, l'acétate de vinyle, le valérate de vinyle ou le propionate de vinyle.

3. Méthode selon la revendication 1, dans laquelle ladite enzyme est immobilisée sur un support.

4. Méthode selon la revendication 1, dans laquelle le substrat est le 2-N-benzyloxycarbonyl(Z)sérinol, le glycéridol, le solkétal, le (±)2-octanol, le sulcatol ou le ferrocényléthanol.

5. Méthode selon la revendication 1, dans laquelle ladite protéase est immobilisée sur un support.

6. Méthode selon la revendication 4, dans laquelle le réactif acylant est l'acétate de vinyle ou le propionate de vinyle.

7. Méthode selon la revendication 1, dans laquelle le réactif acylant est l'acétate de vinyle ou l'acétate d'isopropényle.

8. Méthode selon une quelconque des revendications 1 à 7, dans laquelle la protéase est la protéase N.