Perkin 1 Abstracts: Biocatalysis in Organic Synthesis

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Perkin 1 Abstracts: Biocatalysis in Organic Synthesis aims to cover recent literature concerning the applications of enzymes and micro-organisms as catalysts in organic synthesis. The abstracts will emphasise the key synthetic step(s) that are mediated by the biocatalyst. Emerging technologies for biocatalyst design and optimisation will also be included.

Chemoenzymatic synthesis of glycopolymers OH OH COOH OH

$$\begin{array}{c} \text{HO} \\ \text{NHAC} \\ \text{NHAC} \\ \text{H} \\ \text{O} \\ \text{NHAC} \\ \text{H} \\ \text{O} \\ \text{O} \\ \text{H} \\ \text{O} \\ \text{O} \\ \text{O} \\ \text{O} \\ \text{H} \\ \text{O} \\ \text{O} \\ \text{O} \\ \text{O} \\ \text{H} \\ \text{O} \\$$

(a) UDP-Gal, α -lactalbumin, β -(1,4)-galactosyl transferase, HEPES buffer pH 6.5, 37 °C, 72 h: 100% yield, 100% galactosylation.

(b) CMP-NANA, α-(2,3)-sialyl transferase, sodium cacodylate buffer pH 6, 0.1% Triton X-100, 37 °C, 5 d: 75% yield, 100% sialylation. Then GDP-Fucose, α-(1,3)-fucosyl transferase V, HEPES buffer pH 7.5, NaN₃ MnCl₂•4H₂O, 37 °C, 3 d: 21% yield, 50% fucosylation.

Other alvonolymer

Other glycopolymers were screened for galactosylation. Hydrophilic spacers (S) gave reduced enzymatic galactosylation efficiency. From the results, it can be assumed that the sugar density, structure and flexibility of the spacer are the most important parameters for enzyme efficiency.

F. Sallas and S.-I. Nishimura, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2091. Chemoenzymatic synthesis of sequential glycopeptides

(a) UDP-Gal, α -lactalbumin, β -(1,4)-galactosyl transferase, MnCl₂, MgCl₂, dithiothreitol, sodium cacodylate buffer pH 7.5, 37 °C, 72 h: 84% yield, 100% galactosylation.

(b) CMP-NANA, α -(2,3)-sialyl transferase, sodium cacodylate buffer pH 6.0, 0.1% Triton X-100, 37 °C, 5 d. then GDP-fucose, α -(1,3)-fucosyl transferase V, HEPES buffer pH 7.5, NaN₃ MnCl₂-4H₂O, 37 °C, 3 d: 75% yield, 70% sialylation.

F. Sallas and S.-I. Nishimura, J. Chem. Soc., Perkin Trans. 1, 2000, 2091.

Chemoenzymatic synthesis of L-phosphaserine and L-phosphaisoserine

Lipase

OO

$$N_3 = P(O^i Pr)_2 = P(O^i Pr$$

F. Hammerschmidt, W. Lindner, F. Wuggenig and E. Zarbl, *Tetrahedron: Asymmetry*, 2000. 11, 2955.

(R)- and (S)-1 are intermediates in the synthesis of L-phosphaisoserine and L-phosphaserine.

Desymmetrization of meso-cyclopent-2-ene-1,4-diyl 1,4-diacetate

Trichosporon beigelii

U. R. Kalkote, S. R. Ghorpade, R. R. Joshi, T. Ravindranathan, K. B. Bastawade and D. V. Gokhale, *Tetrahedron: Asymmetry*, 2000, 11, 2965.

Improved enzyme efficiency was achieved through addition of ethanol (10% v/v) although high concentrations of ethanol (>50%) were inhibitory for the reaction.

Resolution of (E)- γ -hydroxy- α , β -unsaturated p-tolylsulfoxides

Lipase

$$\begin{array}{c} \text{H}_{3}\text{C} \\ \text{OH} \\ \text{(\pm)} \end{array} \begin{array}{c} Pseudomonas \ cepacia \ lipase \\ \hline ^{i}\text{Pr}_{2}\text{O}, \ vinyl \ acetate,} \\ \text{(E)} \end{array} \begin{array}{c} \text{H}_{3}\text{C} \\ \hline \text{OH} \\ \text{molecular \ sieves, 0.5 \ d} \\ \text{(R_{s}, S_{c}) - 49\% \ yield, 98\% \ d.e.} \end{array} \begin{array}{c} \text{O} \\ \text{F} \\ \text{OH} \\ \hline \text{OAc} \\ \hline \text{OAc} \\ \hline \text{OAc} \\ \hline \end{array} \begin{array}{c} E > 50 \\ \text{OAc} \\ \hline \text{OAc} \\ \hline \end{array}$$

V. G. de la Rosa, M. Ordóñez and J. M. Llera, *Tetrahedron: Asymmetry*, 2000, **11**, 2991.

Various γ -analogues (${}^{i}Pr$, ${}^{f}Bu$, Pent, Et) were screened to study the reactivity and diastereoselectivity of the enzymatic reaction.

Discovery of a novel tyrosine-2,3-aminomutase

2,3-Aminomutase

HO
$$S$$
-(1) S -(2) S -(3) NH_2 N

P. Spiteller, M. Rüth, F. von Nussbaum and W. Steglich, *Angew. Chem., Int. Ed.*, 2000, **39**, 2754.

Labelling studies were used to determine the biosynthetic pathway of the mushroom *Cortinarius violaceus*. Tyrosine-2,3-aminomutase was identified which is the first aminomutase to be detected in fungus. Additional labelling studies confirmed that β -tyrosine (2) was not formed through an addition-elimination mechanism.

Synthesis of fluorinated sugar nucleotides and their use as mechanistic probes

Glycosyltransferase

M. D. Burkart, S. P. Vincent, A. Düffels, B. W. Murray, S. V. Ley and C.-H. Wong, *Bioorg. Med. Chem.*, 2000, **8**, 1937.

Three fluorinated sugars (1-3) were synthesised. A one-pot procedure for the synthesis of the UDP-2-deoxy-2-fluoro galactose (3) which utilised galactokinase, acetate kinase, then galactose-1-phosphate uridyltransferase, pyrophosphorylase and pyrophosphatase was reported. These compounds were used to investigate the mechanism of action of fucosyltransferases, sialyltransferase and galactosyltransferase.

Chemical modification of Subtilisin Bacillus lentus (SBL)

Hydrolase

Subtilisin *Bacillus lentus* cysteine mutants were subjected to chemical modification. The effectiveness of the resulting modified mutants as amidases and esterases was investigated. Various R groups were examined as chiral modifiers. The most remarkable change in the enzyme activity was observed for the R group shown, modifying the N62C mutant *e.g.* esterase activity was 5.4 fold better than the wild type.

M. Dickman and J. B. Jones, Bioorg. Med. Chem., 2000, 8, 1957.

Preparation of (S)-Atenolol and (S)-Propranolol

Rhizopus arrhizus / Geotrichum candidum

Ar OH
$$_{0}$$
 $_{0}$ $_$

S. V. Damle, P. N. Patil and M. M. Salunkhe, Bioorg. Med. Chem., 2000, 8, 2067.

Novel preparation of (S)-Atenolol (2a) and (S)-Propranolol (2b) using the fungi Rhizopus arrhizus and Geotrichum candidum. The advantage over previous synthesis is the simplicity of the reaction. The organisms also metabolise the (R)-acetates but with lower enantioselectivity.

Alcohol dehydrogenase is active in supercritical carbon dioxide

Dehydrogenase

Where
$$R^1 = \sqrt{\frac{r^2}{r^2}}$$
 and $R^2 = Me$ Yield = 81% >99% e.e

Various ketones were studied giving yields in the range 11 to 96% and e.e. from 96 to >99%. The effect of fluorine substitution at the *ortho*, *para* and α position was also studied. Substitution *ortho* and α increased the yield while *para* substitution led to decreased yields.

T. Matsuda, T. Harda and K. Nakamura, Chem. Commun., 2000, 1367.

Enantioselective desymmetrization of prochiral 2,2-disubstituted propane-1,3-diols and *meso*-1,2-diols using 1-ethoxyvinyl 2-furoate

Lipase

Where n = 2: yield = 77%, 97% e.e. Where n = 1: yield = 82%, 55% e.e.

S. Akai, T. Naka, T. Fujita, Y. Takebe and Y. Kita, Chem. Commun., 2000, 1461

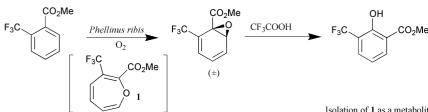
Several other examples are given for congested diols (yields range from 35 to 93%, 61 to 92% e.e.) An improvement in optical purity was observed by prolonging the reaction time and hydrolysing any diester formed.

Where $R^1 = Ph$ and $R^2 = Me$ yield = 66% >99% e.e.

Isolation of stable benzene oxide and evidence for an NIH shift of the carboxymethoxy group during hydroxylation of methyl benzoates

3 eq. of 1-ethoxyvinyl 2-furoate wet Prⁱ₂O, 30 °C

Phellinus ribis

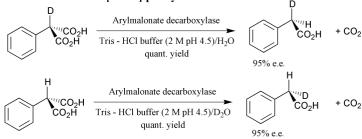


D. R. Boyd, J. T. G. Hamilton, N. D. Sharma, J. S. Harrison, W. C. McRoberts and D. B. Harper, *Chem. Commun.*, 2000, 1481.

Isolation of ${\bf 1}$ as a metabolite provides evidence that o-hydroxylation of methyl benzoates proceeds via benzene oxide intermediates. This is the first report of a hydrogen atom migration from the hydroxylation site and its retention at an adjacent carbon atom ('NIH shift').

Synthesis of both enantiomers of $[\alpha^{-2}H]$ phenylacetic acid

Decarboxylase



K. Matoishi, S.Hanzawa, H. Kakidani, M. Suzuki, T. Sugai and H. Ohta, *Chem. Commun.*, 2000, 1519.

Arylmalonate decarboxylase (EC. 4. 1. 1. 76) was obtained by overexpression in *E.coli* JM 109.

Kinetic resolution of racemic aldols

Ab 38C2

PBS
toluene, 88 h, r.t.

Ab 84G3

PBS
toluene, 340 h, r.t.

PBS = phosphate buffered saline

J. M. Turner, T. Bui, R. A. Lerner, C. F. Barbas III and B. List, *Chem. Eur. J.*, 2000, **6**, 2772.

OH O Aldolase antibodies

OH O (R)-49% recovery, >97% e.e.

A multigram-scale *retro*-aldol kinetic resolution of racemic aldols was developed using commercial catalytic antibodies. The resolution offers a number of advantages over previous methods, namely lower reaction volumes, the ability to recycle the catalyst and mild reaction conditions. The scope of the reaction was examined and other racemic aldols were resolved with yields of 44-50% and e.e.'s of 97-99%.

Asymmetric reduction of enones

R = Me, Et, Pr

 $R = Me, Et, Pr \label{eq:R}$ T. Hirata, K. Shimoda and T. Gondai, *Chem. Lett.*, 2000, 850.

Nicotiana tabacum

$$R = H \text{ Et}$$
 $R = M \text{ Pr}$
 $R' = M \text{ Pr}$

Three enone reductases, p44, p74 and p90, isolated from *Nicotiana tabacum*, were used to asymmetrically reduce the C-C double bond of enones. Complementary reactivity was observed, with both *endo-* and *exo-*cyclic double bonds being reduced in moderate to high e.e. (75-99%) and yield (35-99%).

Asymmetric hydroxylation of unactivated alkanes

Monooxygenase

$$OH \qquad (S) > 99\% \text{ e.e.} \qquad OH \qquad (S) > 99\% \text{ e.e.} \qquad OH \qquad (S) > 99\% \text{ e.e.} \qquad (S) > 99\% \text{$$

W. Adam, Z. Lukacs, C. R. Saha-Möller, B. Weckerle and P. Schreier, Eur. J. Org. Chem., 2000, 2923.

n-alkanes (n = 1 - 4) and cycloalkanes (n = 1 and 3) were subjected to biohydroxylation by B. megaterium. Overoxidation to the corresponding ketone was observed to increase with n, and more markedly for the cyclic systems.

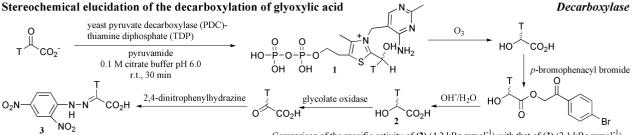
Lipase resolution of α -methylene β -lactams

Lipase

R² Chirazyme L-2 lipase from Candida antarctica
$$H_2O$$
, 70 °C R^1 NH R^2 NH R^2 NH_2 NH_2

W. Adam, P. Groer, H.-U. Humpf and C.R. Saha-Möller, *J. Org. Chem.*, 2000, **65**, 4919.

The β -lactams were resolved using lipase L-2. Three substrates, with variations in R^1 and R^2 were tested. Other enzymes tested were found to be less effective.



 $R^1 = Et$, $R^2 H$

Comparison of the specific activity of (2) (4.2 kBq mmol⁻¹) with that of (3) (2.1 kBq mmol⁻¹) indicated that half of the tritium label had been lost from (2) and it was concluded that (1) was H. Vegad, M. Lobell, S. Bornemann and D. H. G. Crout, *J. Chem. Soc.*, racemic. Several explanations are discussed with reference to models for the mechanism of

Perkin Trans. 1, 2000, 2317. Chem. Soc., racemic. Several explanations are discussed with reference to models for the pyruvate decarboxylase.

Chemoenzymatic synthesis of a biotin-labelled glycophosphononapeptide

Acylase

T. Kappes-Roth and H. Waldmann, J. Chem. Soc., Perkin Trans. 1, 2000, 2579

Intermediate in the synthesis of a glycophosphononapeptide of the c-Myc oncoprotein.

Asymmetric reduction of ethynyl ketones and ethynylketoesters

Secondary alcohol dehydrogenase (SADH)

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50 mM Tris buffer pH 8.0.

$$\frac{us}{\int_{n}^{\infty}}$$
 OR

(R)-1 - 88% yield, >98% e.e.

$$R = CH(CH3)2$$

$$R$$

$$R = CH(CH3)2$$

50 °C, 1 h

SADH from Thermoanaerobacter ethanolicus

(S)-2 - 50% yield, >98% e.e.

C. Heiss and R. S. Phillips, J. Chem. Soc., Perkin Trans. 1, 2000, 2821.

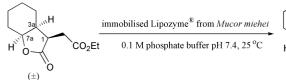
A variety of R groups were screened for both substrates with varying success. Larger ethynyl ketones $\mathbf{2}$ (e.g. R = CH₂CH(CH₃)₂) were reduced to afford the complementary (R)-alcohols.

Resolution of $(1R^*, 3aS^*, 7aS^*)$ -hexahydro-2-oxobenzofuran-3-ylacetic acid ethyl ester

50 mM Tris buffer pH 8.0,

50 °C, 2 h

Lipase



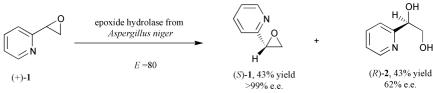
$$H$$
 H
 CO_2Et
 H
 CO_2H
 $E = 13$
 $(+)$ - $(1S,3aR,7aR)$
 $(-)$ - $(1R,3aS,7aS)$
 $(-)$ -

At higher conversion values (64%) the (+)-ester was obtained in 30% yield with 98% S. Drioli, F. Felluga, C. Forzato, G. Pitacco and E. Valentin, *J. Chem. Soc., Perkin* e.e. Other lipases were screened. Horse liver acetone powder (HLAP) showed *Trans. 1*, 2000, 2839.

At higher conversion values (64%) the (+)-ester was obtained in 30% yield with 98% e.e. Other lipases were screened. Horse liver acetone powder (HLAP) showed enantiocomplementary activity with respect to Lipozyme.

Preparation of enantiopure (S)-2-pyridyloxirane

Epoxide hydrolase



Y. Genzel, A. Archelas, Q. B. Broxterman, B. Schulze and R. Furstoss, *Tetrahedron: Asymmetry*, 2000, **11**, 3041.

(S)-Pyridyloxirane 1 was resolved using *Aspergillus niger* epoxide hydrolase (AnEH). Enzymatic attack occurs almost exclusively at the β -carbon of each enantiomer of the substrate, resulting in retention of configuration in the diol product.

Optimisation of lipase-catalysed hydrolysis of meso-oxiranedimethanol

Lipase

J. D. Moseley and J. Staunton, Tetrahedron: Asymmetry, 2000, 11, 3197.

The butyrate ester was tested against a range of lipases (yields range from 13 to 39%, 6 to 84% e.e.) and optimisation of physical parameters was reported.

Resolution of an α -methylene- γ -lactonic ester

Lipase

Porcine pancreatic lipase (PPL)

buffer, 10% acetone 37% conversion

$$(-)-(R)-1$$
 $(+)-(S)-2$

89% e.e.

G. Pitacco, A. Sessanta o Santi and E. Valentin, *Tetrahedron: Asymmetry*, 2000, 11, 3263.

The enantiocomplementary resolution to the one illustrated was performed using *Candida rugosa* lipase to yield (–)-(R)-2 with 82% e.e. Sequential resolutions of optically active acids yielded e.e.s of 97% and 99% for (–)-(S)-2 and (–)-(R)-2 respectively.

Synthesis of chiral cyclopropanes

Rhodococcus sp.

$$\begin{array}{c} & & \\ & & \\ \text{Ar} & \\ & & \\ \text{CN} & \\ & & \\ \text{phosphate buffer, pH 7.0, 30 °C} \end{array}$$

$$\begin{array}{c} \\ \text{phosphate buffer, pH 7.0, 30 °C} \end{array}$$

$$\begin{array}{c} \\ \text{Ar} & \\ \text{C}_{6}H_{5} & \\ \text{4-Me-C}_{6}H_{4} & \\ \text{4-F-C}_{6}H_{4} & \\ \text{4-MeO-C}_{6}H_{4} & \\ \text{4-MeO-C}_{6}H_{4} & \\ \text{th} & \\ \end{array}$$

M.-X. Wang and G.-Q. Feng, Tetrahedron Lett., 2000, 41, 6501.

$$I_2NOC_{ij_2}$$
 Ar Ar

(-)-(1R, 2R)-2

The nitrile hydratase activity of *Rhodococcus* sp. AJ270 was not stereoselective, but the amidase activity yielded aryl amides in up to 50% yield with >99% e.e. This was not the case where $Ar = 4\text{-MeOC}_6H_4$, where the selectivity was poor. Carboxylic acids were obtained in yields of up to 53% with e.e.s of up to 71%.

Asymmetric reduction of ketones by a cyanobacterium

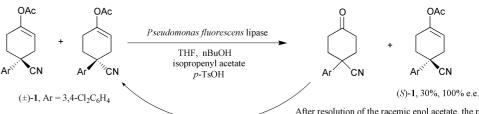
Synechococcus sp.

K. Nakamura, R. Yamanaka, K. Tohi and H. Hamada, Tetrahedron Lett., 2000, 41, 6799.

Synechococcus is a phototrophic cyanobacterium, which requires light and carbon dioxide to grow and perform transformations. In addition to the pentafluoroacetophenone 1 shown, a series of o-, m-, and p- substituted acetophenones are also accepted as substrate, where the substituent is F, Cl, Me and OMe. E.e.s of alcohols obtained were in the range 96-100%.

Resolution of an enol acetate

Lipase



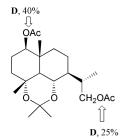
A. J. Carnell, M. L. Escudero Hernandez, A. Pettman and J. F. Bickley, Tetrahedron Lett., 2000, 41, 6929.

After resolution of the racemic enol acetate, the product ketone is recycled. After 68% conversion to ketone, enol acetate in 30% yield with 100% e.e. was recovered. This synthon was then used in a synthesis of a non-peptide tachykinin NK-2 antagonist.

Biotransformation of eudesmane sesquiterpenes

D, 8% H 60% ŌАс CH₂OH Î

D, 30%



A, García-Granados, E. Melguizo, A. Parra, F.L. Pérez, Y. Simeó, B. Viseras and J. M. Arias, Tetrahedron, 2000, 56, 6517.

Rhizopus nigricans D, 40% Ŋ OAc deacetylation, 5% ҈ОН ¦ ĊH₂OAc R, 10%

D, 25% ОН ŌАс CH₂OAc D, 30%

A series of eudesmane sesquiterpenes was prepared by semisynthesis from vulgarin and used as substrates for biotransformation by $Rhizopus\ nigricans$. In addition to regioselective deacetylation (D), hydroxylations (H) and reductions (R) were observed, yielding a series of hydroxyselinane derivatives.