Organic Process Research & Development **2002**, *6*, 463–470

Rapid Determination of Enantiomeric Excess Using Infrared Thermography

Nicolas Millot, Phil Borman, Mike S. Anson, Ian B. Campbell, Simon J. F. Macdonald, and Mahmoud Mahmoudian*

GlaxoSmithKline Research and Development, Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, England

Abstract:

Infrared thermography (IRT) is presented as a novel technique to screen a potentially large number of asymmetric catalysts or substrates in a high-throughput fashion. IRT was used as a simple, rapid, and practical approach for initial screening of the substrate specificity of *Candida antarctica* lipase. This was carried out using a 96-well microtitre plate format. Potential advantages and limitations of IRT for the enzymatic stereoselective acylation of primary and secondary alcohols of interest are discussed.

Introduction

Enzymes have been used extensively in chemical synthesis to generate chiral synthons.[1,2] Enzymatic kinetic resolution is one of the most widely used approaches for the preparation of chiral pharmaceutical intermediates as enantiomers of racemic mixtures can often be efficiently resolved in this manner.[3] Rapid determination of enantiomeric excess (ee) is frequently hampered by the availability of a suitable chiral assay. Chiral HPLC, GC, and chiral shift NMR are the most preferred techniques, but these methods can suffer from being too time-consuming to develop. Other approaches used by several groups[4,5] include fluorescence,[6] mass spectroscopy,[7] capillary electrophoresis,[8] and circular dichroism[9] techniques.

Among these methods, infrared thermography (IRT) has attracted considerable interest in recent years because noninvasive thermal imaging of chemical reactions can be performed through detection of emitted infrared radiations.[10] *In vitro* and *in vivo* biological processes have also been detected by IRT as small temperature changes can be measured in cellular metabolism, growth, and toxic response.[11] Acceleration of drug screening is one of the potential applications of IRT in the pharmaceutical industry.

Given the recent reports from Reetz et al. on the use of IRT for the selection of chiral catalysts,[12] we decided to extend these studies and to investigate its potential application for the rapid quantification of ee in enzyme-catalysed reactions.[13,14]

Results and Discussion

We at GlaxoSmithKline frequently screen biocatalysts (isolated enzymes, recombinant microorganisms) and chiral ligands for enantioselective transformations. For high-throughput screening (HTS), the bottleneck is usually in the speed of chiral analyses involving laborious development of chiral HPLC and GC assays. Consequently, we are particularly interested in developing new approaches for rapid determination of ee. Recently Reetz et al. highlighted the use of thermal imaging for the screening of enantioselective biocatalysts.[12] Interestingly, they observed a differential heat output during the thermal imaging of lipase-catalysed acy-

* Author for correspondence. E-mail: mm6382@yahoo.co.uk.

(1) Koeller, K. M.; Wong, C.-H. Enzymes for Chemical Synthesis. *Nature* **2001**, *409*, 232.

(2) Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolts, M.; Witholt, B. Industrial Biocatalysis Today and Tomorrow. *Nature* **2001**, *409*, 258.

(3) Keith, J. M.; Larrow, J. F.; Jacobsen, E. N. Practical Considerations in Kinetic Resolution Reactions. *Adv. Synth. Catal.* **2001**, *343*, 5.

(4) For excellent reviews see: (a) Reetz, M. T. Combinatorial and Evolution-Based Methods in the Creation of Enantioselective Catalysts. *Angew. Chem., Int. Ed.* **2001**, *40*, 284. (b) Wahler, D.; Reymond, J.-L. Novel Methods for Biocatalyst Screening. *Curr. Opin. Chem. Biol.* **2001**, *5*, 152.

(5) (a) Le Bars, J.; Häussner, T.; Lang, J.; Pfaltz, A.; Blackmond, D. G. A Scale-Transparent Reaction Calorimetric Assay for Rapid Catalyst Screening. *Adv. Synth. Catal.* **2001**, *343*, 207; (b) Jandeleit, B.; Schaefer, D. J.; Powers, T. S.; Turner, H. W.; Weinberg, W. H. Combinatorial Material Science and Catalysis. *Angew. Chem., Int. Ed.* **1999**, *38*, 2494.

(6) (a) Korbel, G. A..; Lalic G.; Shair, M. D. Reaction Microarrays: A Method for Rapidly Determining the Enantiomeric Excess of Thousands of Samples *J. Am. Chem. Soc.* **2001**, *123*, 361. (b) Badalassi, F.; Wahler, D.; Klein, G.; Crotti, P.; Reymond, J.-L. A Versatile Periodate-Coupled Fluorogenic Assay for Hydrolytic Enzymes. *Angew. Chem., Int. Ed.* **2000**, *39*, 4067. (c) Reetz, M. T.; Sostmann, S. 2,15-Dihydroxyhexahelicene (HELIXOL): Synthesis and Use as an Enantioselective Fluorescent Sensor *Tetrahedron* **2001**, *57*, 2515.

(7) Reetz, M. T.; Becker, M. H.; Klein, H.-W.; Stöckigt, D. A Method for High-Throughput Screening of Enantioselective Catalysts. *Angew. Chem., Int. Ed.* **1999**, *38*, 1758.

(8) Reetz, M. T.; Kühling, K. M.; Deege, A.; Hinrichs, H.; Belder, D. Super-High-Throughput Screening of Enantioselective Catalysts by Using Capillary Array Electrophoresis *Angew. Chem., Int. Ed.* **2000**, *39*, 3891.

(9) Ding, K.; Ishii, A.; Mikami, K. Super High-Throughput Screening (SHTS) of Chiral Ligands and Activators: Asymmetric Activation of Chiral Diol-Zinc Catalysts by Chiral Nitrogen Activators for the Enantioselective Addition of Diethylzinc to Aldehydes *Angew. Chem., Int. Ed.* **1999**, *38*, 497.

(10) Taylor, S. J.; Morken, J. P. Thermographic Selection of Effective Catalysts from an Encoded Polymer-Bound Library *Science* **1998**, *280*, 267.

(11) (a) Paulik M. A.,; Buckholz, R. G.; Lancaster, M.; E. Dallas, W. S.; Hull-Ryde, E. A.; Weiel, J. E.; Lenhard, J. M. Development of Infrared Imaging to Measure Thermogenesis in Cell Culture: Thermogenic Effects of Uncoupling Protein-2, Troglitazone, and Adrenoceptor Agonists. *Pharm. Res.* **1998**, *15*, 944. (b) Lenhard, J. M.; Paulik, M. A. Infrared Thermography for Measuring Real-Time Thermogenesis in Organisms and Cells. PCT Int. Appl. WO 9960630 A1 19991125, 1999.

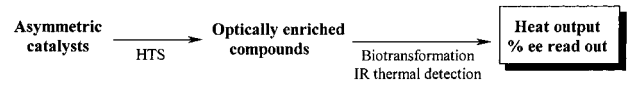
(12) (a) Reetz, M. T.; Becker, M. H.; Kühling, K. M.; Holzwarth, A. Time-resolved IR-Thermographic Detection and Screening of Enantioselectivity in Catalytic Reactions. *Angew. Chem., Int. Ed.* **1998**, *37*, 2647. (b) Reetz, M. T.; Becker, M. H.; Liebl, M.; Fürstner, A. IR-Thermographic Screening of Thermoneutral or Endothermic Transformations: The Ring-Closing Olefin Metathesis Reaction. *Angew. Chem., Int. Ed.* **2000**, *39*, 1236.

(13) High-throughput enzyme−inhibitor screening has been reported by an array of thermistors: Connolly, R.; Sutherland, J. D. Catalyst Screening Using an Array of Thermistors. *Angew. Chem., Int. Ed.* **2000**, *39*, 4268.

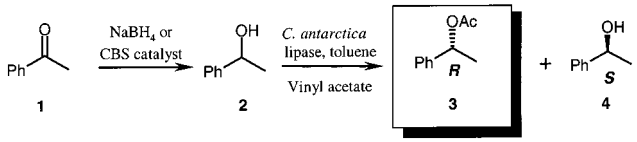
(14) Low-throughput calorimetric methods have been reported for monitoring enzymes reactions: (a) Ramsden, D. K.; Webster, N. A.; Peron, Y.; Hughes, J. Calorimetric Method for the Monitoring and Control of Bioreactions. *Biotechnol. Lett.* **2000**, *22*, 1259. (b) Stefuca, V.; Gemeiner, P. Investigation of Catalytic Properties of Immobilized Enzymes and Cells by Flow Microcalorimetry. *Adv. Biochem. Eng. Biotechnol.* **1999**, *64*, 69 and references therein.

10.1021/op010093k CCC: $22.00
Published on Web 03/05/2002

Scheme 1. **HTS of enantioselective catalysts with infrared IRT**



Scheme 2. **Sodium borohydride ($NaBH_4$) and CBS reduction of 1 followed by enzymatic kinetic resolution of 2 catalysed by immobilised *C. antarctica* lipase[a]**



[a] $NaBH_4$ reduction of **1** affords *Rac*-**2** (chiral HPLC) whereas, CBS reduction affords *R*-**2** (86% ee by chiral HPLC).

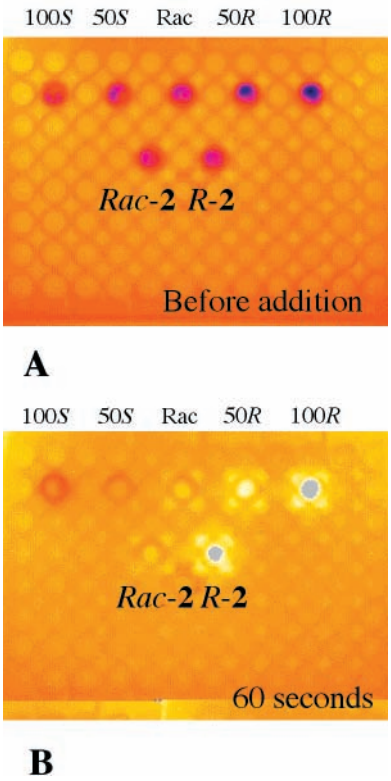


Figure 1. **Time-resolved thermal imaging of lipase-catalysed enantioselective acylation of 1-phenylethanol, 2. Colours correspond to the following temperatures. (A) At time zero; black (12.5−13.5 °C), purple (15−16 °C), pink (17−18 °C), orange (18.5−19.5 °C). (B) After 60 s; orange (18.5−19 °C), yellow (19.5−20 °C), white (20.5 °C), grey (21.4−21.6 °C).**

lation of chirally pure *R*- and *S*-alcohols.[12a] We therefore, envisaged a high-throughput chiral screening approach based on thermal detection of enzyme-catalysed kinetic resolutions of racemic mixtures and proceeded to develop this further (Scheme 1).

We chose to study the lipase-catalysed acylation of 1-phenylethanol, **2**, with *Candida antarctica* as a model system (Scheme 2). Reactions were carried out in a 96-well microtitre plate containing solutions of **2** and the immobilised lipase. Standard solutions of various mixtures of optically pure *R*- and *S*-**2** (0−100% *R* or *S*-isomer) were used for determining ee values (first row, Figure 1). Solutions of *Rac*-**2** (prepared by sodium borohydride reduction of acetophenone **1**) and *R*-**2** (86% ee, prepared by CBS reduction of **1**) were added to the second row of the plate (Figure 1A). Temperature changes were monitored periodically with an infrared camera (thermal imaging), and reactions were started by adding solutions of vinyl acetate in toluene to each well.

Before reactions were initiated, cold spots (blue colour) were evident due to the endothermic process of solvent evaporation (Figure 1A). Subsequently, we observed differential heat outputs (yellow colour) corresponding to various concentrations of the *R*-enantiomer (Figure 1B). This compared well with previous reports (Reetz et al.[12a]) that temperature changes are affected by substrate concentrations. Similarly, wells containing either *Rac*-**2** or *R*-**2** (prepared by chemical reductions) showed elevated heat outputs (Figure 1B). *C. antarctica* lipase acylation of *Rac*-**2** has been shown to be *R*-enantioselective (i.e. the *R*-isomer is preferentially acylated leaving behind the corresponding *S*-isomer, Scheme 2).[12] This suggested that an excellent *R*-enantioselectivity was also obtained after CBS reduction[15] of **1** (86% ee by chiral HPLC), Figure 1. These results therefore, gave us encouragement to further investigate the use of infrared thermal imaging for rapid determination of ee.

To quantify temperature changes from enzymatic reactions, heat output was measured as a function of time (Figure 2). Initially, during the first 9 s of each reaction, cooling was observed as a result of the addition of a solution of vinyl acetate.[16] Temperature changes were observed almost immediately in each well after reactions had started.[17] Furthermore, there was a noticeable differential heat output corresponding to the various mixtures of optically pure *R*- and *S*-**2** (black curves, Figure 2). Indeed, the observed order of heat output corresponded to different concentrations of the *R*-enantiomer. We were also encouraged to observe that, at given concentrations of alcohol, lipase, and vinyl acetate, the heat output generated during reaction fitted perfectly on the standard curves (black curves), data not shown. The calculated ee for racemic alcohol **2** was found to be near zero and 75−100% ee for enantiomerically enriched alcohol *R*-**2** (Figure 2).[18] This corresponded well with the previously observed values of zero and 86%, respectively, as analysed by chiral HPLC. This therefore, demonstrated the proof-of-principle that IRT was a viable approach for use in HTS of asymmetric catalysts.

With these promising results in hand we decided to investigate this further to see if the heat generated during lipase-catalysed resolutions of **2** could be quantified more

(15) Corey, E. J.; Helal, C. J. Reduction of Carbonyl Compounds with Chiral Oxazaborolidine Catalysts: A New Paradigm for Enantioselective Catalysis and a Powerful New Synthetic Method. *Angew. Chem., Int. Ed.* **1998**, *37*, 1986.

(16) Vinyl acetate is a highly volatile compound (bp = 77 °C); consequently, an endothermic process is observed by IRT due to evaporation of this reagent.

(17) We believe the heat observed during the first stages of the lipase-catalysed reactions is partly due to the mixing enthalpy of the highly concentrated solutions (exothermic effect in this case).

(18) The use of emmisivity-corrected IRT did not improve the accuracy of the ee reading. The method consists of subtracting the thermal background before the start of reactions to data gathered during the catalytic reactions. For a leading reference, see: Holzwarth, A.; Schmidt, H.-W.; Maier, W. F. Detection of Catalytic Activity in Combinatorial Libraries of Heterogeneous Catalysts by IR Thermography. *Angew. Chem., Int. Ed.* **1998**, *37*, 2644.

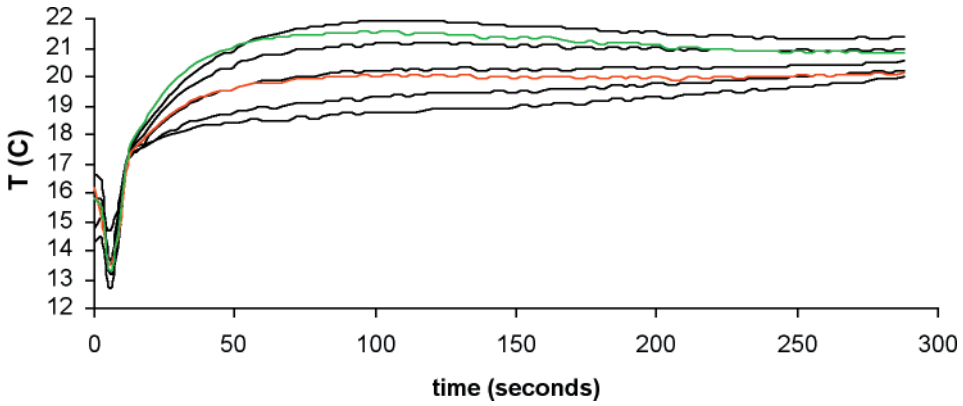


Figure 2. **Time-resolved temperature profile for *C. antarctica* acylation of 1-phenylethanol, 2. Black curves represent first rows (100% *S* − 100% *R*) of each plate (standard curves) as shown in Figure 1. Red and green curves represent reaction wells (second row) containing *Rac*-2 and *R*-2 respectively.**

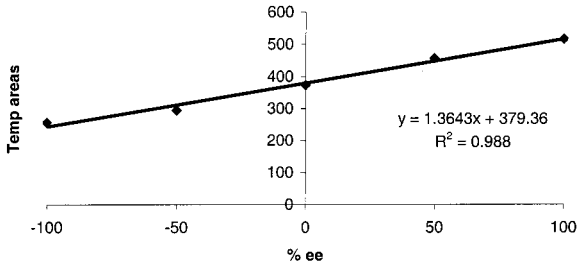


Figure 3. **Correlation between temperature areas and enantiomeric excess for lipase-catalysed resolution of 1-phenylethanol, 2. Positive values of ee were conventionally matched with the *R*-configuration, and negative values, with the *S*-configuration.**

Table 1. **Determination of enantiomeric excess (ee) by IRT[a]**

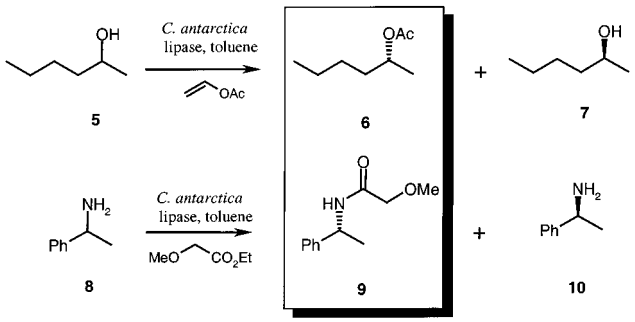
alcohols	Rac-**2**	R-**2**
heat areas (s.K)	368.32	508.93
IRT calculated ee (%)	8	94
chiral HPLC ee (%)	1	86

[a] Temperature areas are expressed as second·Kelvin (s·K)

accurately. We calculated areas under each temperature curve from the start of the reactions (9 s) to the point where the maximum temperature rise was reached (117 s).[19] A linear correlation could be obtained between heat areas and ee values (Figure 3). Plots of areas were fitted to a straight line using the nonweighted linear least-squares method (R^2 = 0.988). This linear equation could be used to calculate of ee values of *Rac*-**2** (−8% ee) and optically enriched *R*-**2** (94% ee). These corresponded very well with ee values previously measured by IRT and chiral HPLC (Table 1).

To extend the scope of this method further, we applied IRT determination of ee to other substrates such as 2-hexanol, **5**, and 1-phenylethylamine, **8**, (Scheme 3). The time-resolved IRT temperature profile for *C. antarctica* at various mixtures of optically pure **5** is shown in Figure 4. The initial rates under each temperature curve gave a good linear correlation

Scheme 3. **Enzymatic kinetic resolutions of 5 and 8 catalysed by immobilised *C. antarctica* lipase**



with the observed ee values (R^2 = 0.9953), data not shown. Similarly, standard curves for the determination of ee by IRT, using solutions of **8** and ethyl methoxyacetate as an acylating agent, were obtained.[20] Temperature areas (s·K), representing solutions of **8** of varying optical purity (100% *S* − 100% *R*), were determined in each row (A−G) of a microtitre plate (Table 2). These gave a good linear correlation with the observed ee values (R^2 = 0.9835) (data not shown). The data were analysed using the statistical software package Minitab.

This experiment was deliberately designed to allow seven different concentrations of optically pure **8** to be analysed simultaneously and was replicated four times (rows A−G) to test for "lack of fit" (Table 2). Lack of fit is observed when a pattern is discernible in the residual plot (residuals are the distances of the experimental points from the fitted regression line, measured in a direction parallel to the response axis). By replicating measurements we can define whether an apparent pattern is significant. This gives information about the inherent variability of the response measurements (called the "pure error"). Unexpectedly, although data from the replicate on row A (at the very edge of the plate) demonstrated a relationship between ee and temperature area, this relationship differed slightly from the other three replicates (Figure 5). This difference in temperature area for row A could be due to its being at the edge of the plate where dissipation of heat may be different from that at the centre of the plate.

(19) The time for which maximum temperature rise is reached (top of temperature curve) is believed to be close to the time for which the reaction is over. For more information, see ref 12a and Simultaneous Assessment of Thermodynamic and Kinetic Behaviour of Chemical Reactions: Theory and Experiment. Davies, G. C.; Hutton, R. S.; Millot, N.; Anson, M. S.; Macdonald, S. J. F.; Campbell, I. B. Manuscript in preparation.

(20) Ditrich, F.; Hauer, K.; Ladner, B. W. Optisch aktive amine durch lipase-katalysierte methoxyacetylierung Backenhohl. *J. Prakt. Chem.* **1997**, *339*, 381.

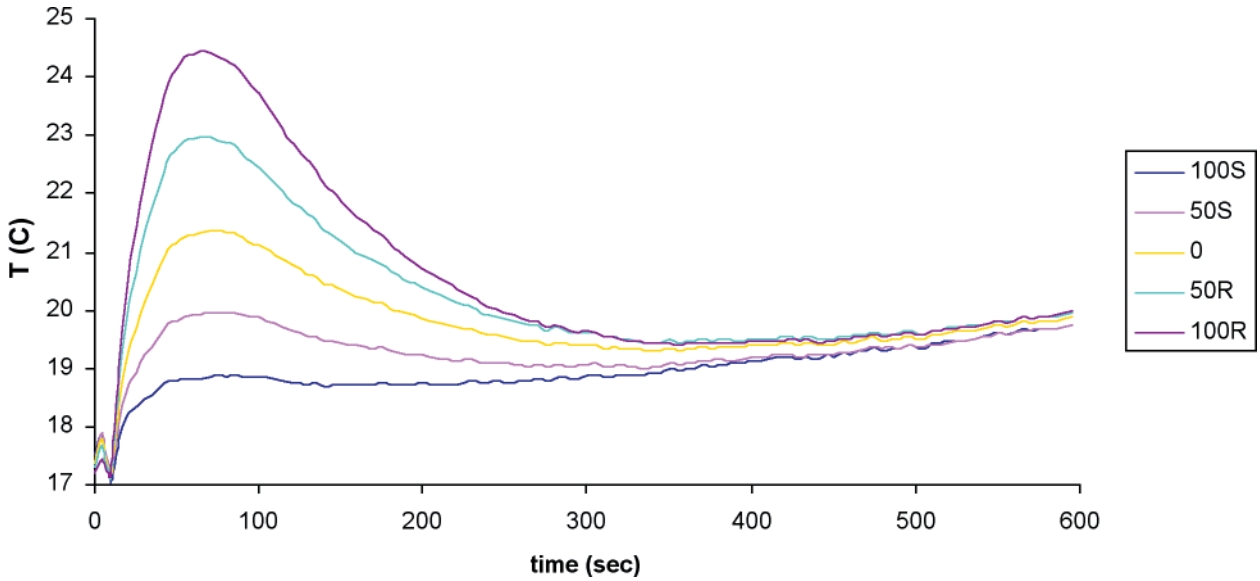


Figure 4. **Time-resolved temperature profile for *C. antarctica* resolution at various mixtures of optically pure 2-hexanol, 5.**

Table 2. **Lipase-catalysed resolution of 8[a]**

ee of **8** in each row	100% (*S*)	75% (*S*)	50% (*S*)	0	50% (*R*)	75% (*R*)	100% (*R*)
rows of plate	*a*	*a*	*a*	*a*	*a*	*a*	*a*
A	369.129	464.148	501.250	603.263	646.656	644.218	691.162
C	395.121	435.323	449.861	549.652	598.866	615.796	628.882
E	401.959	423.204	458.833	513.634	609.919	608.994	636.040
G	401.715	432.426	457.215	526.046	602.104	634.191	654.406

[a] Temperature areas (s·K), representing various mixtures of optically pure **8** [100% (*S*) to 100% (*R*)], were determined in each row (A−G) of a microtitre plate

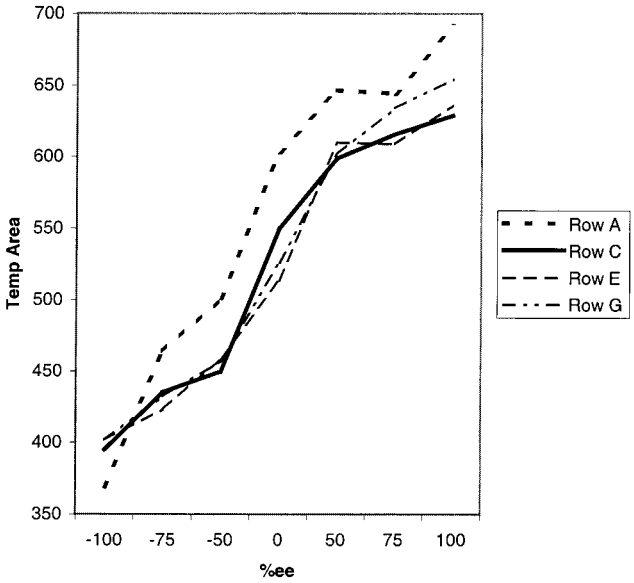


Figure 5. **Relationship between rows, ee, and temperature area as stated in Table 2.**

In the following analysis, to illustrate how the ee can be predicted from temperature area, the data from row A was excluded. It should be noted that, if the cause of the variability of the row A data cannot be determined, then the estimated precision for predicted ee in the following analysis will be an overestimate. A first order polynomial was fitted to the data (rows C, E, and G). The *y*-intercept (the constant) and the linear (*x* term) predictors were found to be highly significant (Probability < 0.0005) to the model. This model

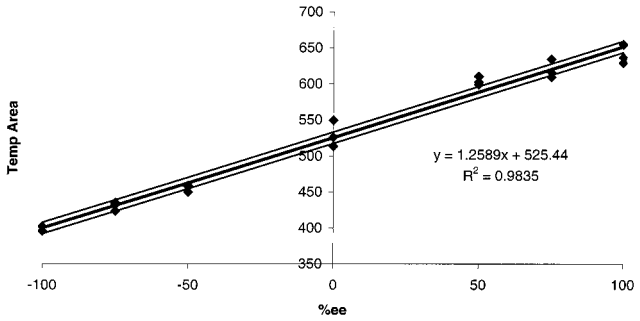


Figure 6. **Linear relationship with 95% confidence intervals between ee and temperature area using row C, E, and G data as stated in Table 2.**

along with confidence intervals at the 95% level were plotted (Figure 6). This can be used to predict an unknown ee from samples with known temperature area to approximately ±5%. There is no significant lack of fit associated with the above model; in other words, the residuals plotted against ee resembled a random sample from a normal distribution with zero mean. A normal probability plot of the residuals also suggested that there was no evidence against normality (The Anderson−Darling normality test produced a probability value of 0.798). The 95% confidence intervals (Figure 6), fitted around the above first order polynomial model, demonstrate very high confidence in the mean temperature area response. To use these intervals as a predictive tool in analysing a sample of unknown ee, at least six replicate experiments should be carried order to achieve a good approximation of the mean temperature area. If, however,

Table 3. **Ninety-six-well microtitre plate showing *C. antarctica*-catalysed acylation of 96 racemic alcohols using IRT[a]**

H	G	F	E	D	C	B	A	
 3.5 ; 54	 5.1 ; 100	 3.4 ; 28	 2.1 ; 52	 1.9 ; 2	 2.4 ; 12	 2.8 ; 0	 6.4 ; 57	1
 2.5 ; 60	 4.8 ; 62	 3.9 ; 44	 2.9 ; 40	 3.9 ; 41	 3.1 ; 72	 3.8 ; 44	 7.1 ; 43	2
 1.9 ; 0	 4.2 ; 99	 4.3 ; 51	 4.5 ; 60	 5.2 ; 60	 4.2 ; 30	 4.5 ; 63	 8.5 ; 7	3
 4.9 ; 54	 4.6 ; 60	 4.9 ; 56	 5.2 ; 62	 5.4 ; 55	 6.2 ; 60	 6.6 ; 58	 5.7 ; 59	4
 1.8 ; 32	 1.7 ; 29	 2.8 ; 30	 2.8 ; 7	 4.3 ; 59	 4.4 ; 47	 4.0 ; 57	 4.0 ; 50	5
3.1 ; 31	2.7 ; 55	3.1 ; 0	2.8 ; 33	3.2 ; 54	2.8 ; 7	3.5 ; 58	4.0 ; 100	6
1.6 ; 6	3.0 ; 60	4.4 ; 61	4.0 ; 83	3.1 ; 20	2.5 ; 80	2.5 ; 0	2.6 ; 0	7
2.1 ; 53	2.1 ; 30	3.0 ; 43	3.5 ; 50	3.4 ; 53	2.9 ; 50	2.5 ; 93	3.2 ; 67	8
2.3 ; 60	6.8 ; 74	3.4 ; 56	3.1 ; 59	2.8 ; 0	2.9 ; 0	3.1 ; 43	3.6 ; 59	9
3.1 ; 40	3.2 ; 40	4.5 ; 63	2.0 ; 62	2.6 ; 65	4.1 ; 68	5.1 ; 55	5.0 ; 100	10
2.7 ; 36	3.4 ; 46	6.0 ; 53	2.4 ; 0	2.2 ; 20	1.8 ; 0	2.7 ; 0	3.4 ; 7	11
3.6 ; 57	2.4 ; 51	4.2 ; 78	2.5 ; 46	1.7 ; 0	2.6 ; 46	3.6 ; 76	2.8 ; 40	12

[a] Numbers (*T*; %) are related to the temperature rise (*T*) measured by IRT and conversions (%) measured by GC after 90 min. A−H and 1−12 represent rows of microtitre plate.

only a rough estimate of ee is required rather than a statistically significant value, a duplicate measurement is probably all that is needed.

To extend the application of IRT to biocatalysis, we turned our attention to the screening of alcohols for acylations catalysed by *C. antarctica* lipase. It is important to note that an initial objective of a HTS campaign is its speed. In other words, the process must rapidly indicate which substrates are worthy of further study. Although desirable, it is unrealistic to expect an HTS process to deliver completely reliable and reproducible data for every substrate.

Ninety-six racemic mixtures of secondary alcohols of interest (Table 3) were screened in a microtitre plate (reaction volume, 0.2 mL). Toluene was chosen as the reaction solvent because of its low volatility. In some cases dioxane was preferred for solubility reasons (alcohols A2, A3, H2, H7, Table 3). Analysis of thermal imaging pictures at regular time intervals showed some surprising results (Figure 7). In particular, the strongest heat output was immediately observed for alcohol G9 having a tertiary amino group in the γ-position (Figure 7).[21] Significant temperature rises were

(21) As a control experiment a reaction in the same conditions has also been run for alcohol G9 without any enzyme catalyst. It was found that no reaction occurred after a prolonged time, suggesting that the heat output observed by IRT was not due to intramolecular catalysis from tertiary amino group but rather by enzyme catalysis.

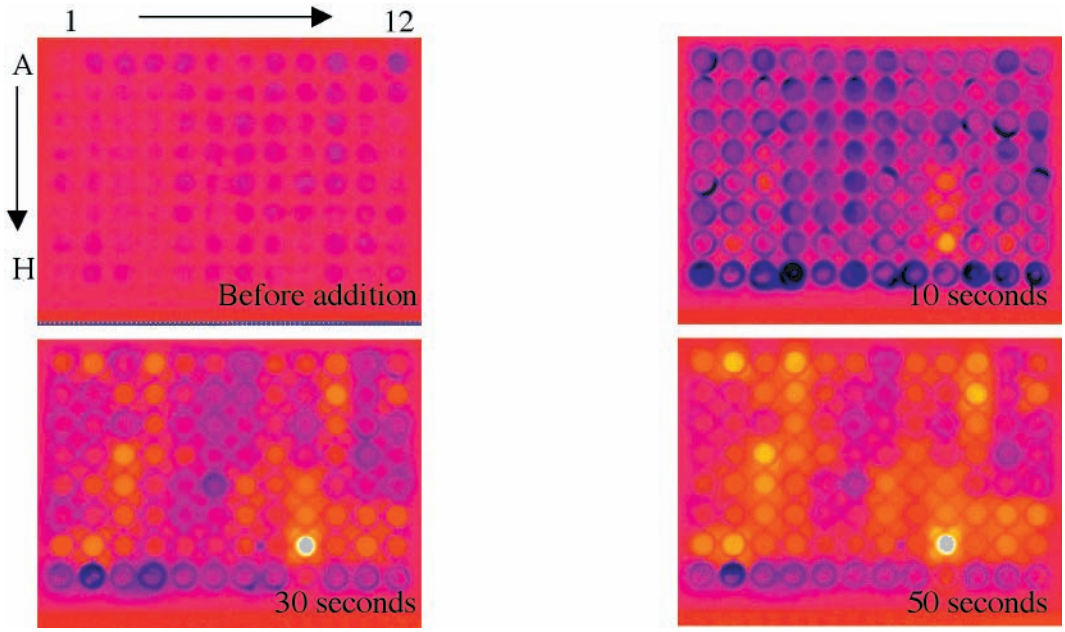


Figure 7. **Time-resolved thermal imaging for screen of lipase-catalysed acylations of 96 alcohols as stated in Table 3. Colours correspond to the following temperatures: black (14−15 °C), purple-blue (16−17 °C), pink-red (19−20 °C), red-orange (20−21 °C), orange-yellow (21.5−22.5 °C), yellow (23−24.5 °C), white (25−26 °C), grey (26−27 °C).**

also seen after 10 s for substrates with a tertiary amino group at the α-position (E9 and F9). For comparison, heat outputs from reactions of similar alcohols not containing any basic functionality (e.g. A4, B4, D4, B12, and G11) were detected much later (Figure 7). This may suggest that the kinetics of acylation of alcohols E9−G9 could be substantially different than that for reactions with alcohols having no basic functionality.

Attempts to obtain a more accurate readout for each well proved to be more tricky as quantification of the generated heat needs to be considered. In fact, we tentatively applied the emissivity-corrected IRT method as previously reported by Maier et al.[18] by subtracting the maximum reaction temperatures measured for each well from initial temperatures measured before addition of the final reactant. It was apparent that a small temperature rise of at least 1.7 °C was detected in each well.[22] To investigate this further, the extents of reactions were determined by GC (Table 3). Interestingly, despite the appearance of small temperature rises, no reaction at all was detected in 11 wells (B1, H3, F6, A7, B7, C9, D9, B11, C11, E11, D12) as judged by GC highlighting a potential limitation of IRT. For example, the highest temperature rise was 3.1°K for a well with no reaction. This was therefore used as the background reading; 46 wells gave a temperature rise under 3.1 K, whereas the remaining 50 wells showed a temperature rise above 3.1 K. This detection rate is typical for HTS where the emphasis is on rapid detection of interesting wells for further study rather than an exhaustive assessment of each substrate. Furthermore, although chiral analyses (chiral GC) of selected wells showed that in some cases acylations were indeed enantioselective (Table 4), this however, did not correspond well with the generated heat outputs.

(22) When an empty plate is placed under the IRT camera, a temperature rise of approximately 2 °C is observed over 1 h. For more information see ref 19.

Table 4. **Enantiomeric ratio (*E*) and temperature rises as observed by IRT after lipase-catalysed reactions of selected alcohols (GC analysis)**

wells	conversion (%)	ee (%)	*E*	IRT (K)
A8	67	0	1.0	3.2
C2	72	33	4.8	3.1
D2	41	24	1.9	3.9
D7	20	72	7.3	3.1
G8	30	7	1.2	2.1
G10	40	30	2.2	3.2
H10	40	52	4.4	3.1

The findings on the substrate specificity of *C. antarctica* lipase for acylations of alcohols are consistent with previously reported data on its active site.[23] Finally, we have highlighted a potential drawback of IRT in detection of the heat output in a 96-well format, and the apparent lack of correlation between the observed heat output and enantioselectivity. However, we believe that IRT can provide a crude but practical initial method, when screening large numbers of catalysts and/or substrates, to rapidly identify a particular structural class that can be further accurately analysed by conventional methods. For example the data generated by IRT for the whole plate (96 substrates) took only 15 min, several orders of magnitude faster than analysis by GC or HPLC.

Experimental Section

General Methods. All chemicals, reagents and compounds used were of highest purity. All IRT experiments were performed in polypropylene microtitre plates having 96 U-shaped wells to allow smooth stirring (well volume, 0.25 mL). Reactions were stirred at 1100 rpm with 5 × 2

(23) Anderson, E. M.; Larsson, K. M.; Kirk, O. One Biocatalyst—Many Applications: The Use of *Candida antarctica* B-Lipase in Organic Synthesis. *Biocatal. Biotransform.* **1998**, *16*, 181.

mm magnetic bars. Empty wells were interspersed between each reaction well to prevent any heat cross-contamination. IRT measurements were carried out under a fume hood without taking any other precautions to enhance sensitivity of the system.

Reaction temperatures were measured with a ThermaCAM SC3000 (Flir Systems AB, Sweden) equipped with a GaAs detector (320 × 240 pixels FPA QWIP resolution) and placed at 35 cm above the top of each microtitre plate (top-viewing camera reading). The detection system was sensitive to IR radiation in the wavelength range 8−9 μm and to temperature changes of 5 mK. 16 frames were taken for every thermal picture. Well Plate Thermal Profiler was used as a computer interface to manipulate data from thermal imaging and temperature changes as a function of time. Where applicable, thermal data were transferred to Microsoft Excel for further manipulations.

Immobilized *C. antarctica* lipase (Novozyme-435) was purchased from Novozymes (Denmark). Chiral HPLC analyses of 1-phenylethanol were carried out with Chiralcel OB-H column (flow rate, 1.0 mL/min; heptane/2-propanol, 90/10; column temperature, 30 °C). Retention times for the *S*- and *R*-isomer were 5.6 and 7.0 min, respectively. Chiral GC analyses were carried out with a Chiraldex β-cyclodextrin permethylated hydroxypropyl column (Astec, 20 m × 0.25 mm) with a temperature gradient of 60−200 °C at 5 °C/min and a 2-min hold at 200 °C. Achiral GC analyses were carried out with a HP-1 cross-linked methyl siloxane column (Agilent Technologies, 10 m × 0.1 mm, 0.4 μm film thickness).

Determination of ee by IRT. Procedure A: 1-Phenylethanol (2) and 2-Hexanol (5). Optically active solutions of **2** or **5**, used as standard curves for ee determinations, were prepared by mixing the appropriate amounts of commercially pure *R*- and *S*-alcohols. Immobilised *C. antarctica* lipase (25 mg/well) and 2 M toluene solutions of alcohol (0.1 mL, 0.2 mmol) were dispensed into each well. The temperature reading was started with an IR camera, and reactions were initiated by the addition of 4 M toluene solution of vinyl acetate (0.1 mL, 0.4 mmol) with a multichannel pipet robot. Addition was completed within 10 s. The experiment was run for 5 min and temperature reading was taken every 3 s with **2**. The experiment was run for 10 min, and temperature readings were taken every 5 s with **5**.

Procedure B: 1-Phenylethylamine (8). Similarly, optically active solutions of **8**, used as standard curves for ee determinations, were prepared by mixing the appropriate amounts of commercially pure *R*- and *S*-**8**. Immobilised *C. antarctica* lipase (25 mg/well) and 4 M toluene solutions of amine (0.1 mL, 0.4 mmol) were dispensed into each well. Temperature reading was started with an IR camera, and reactions were initiated by the addition of 4 M toluene solution of vinyl acetate (0.1 mL, 0.4 mmol). Addition was completed within 10 s. The experiment was run for 10 min, and temperature reading was taken every 5 s.

Synthesis of *Rac*-2 by Borohydride Reduction. Sodium borohydride (2.27 g, 60 mmol) was added portionwise at 0 °C to a solution of acetophenone **1** (5.8 mL, 50 mmol) in ethanol (60 mL). This was stirred for 1 h at 0 °C and then warmed to room temperature for a further 1 h. After the reaction mixture was carefully quenched at 0 °C with an aqueous saturated solution of NH_4Cl (100 mL), the resulting biphasic mixture was extracted with ether (3 × 100 mL). The combined organic layers were dried with brine and magnesium sulphate. After filtration the solution was evaporated under vacuo. The resulting crude oil was purified by flash chromatography on silica gel (cyclohexane:ether, 4:1) to yield 2.3 g of a colourless oil (37%, 0% ee).

Synthesis of *R*-2 by CBS Reduction.[15] To a stirred 1 M toluene solution of *S*-2-methyl-CBS-oxazaborolidine catalyst (5 mL, 5 mmol, 10 mol %) at room temperature was added a 1 M solution of BH_3 THF (5 mL, 5 mmol) under a flow of nitrogen. After cooling to 0 °C, a 3.3 M THF solution of **1** (5.8 mL, 50 mmol) and a 1 M solution of BH_3 THF (25 mL, 25 mmol) were added simultaneously dropwise (addition rate 1 drop/sec). The resulting mixture was stirred at room temperature for a further 30 min, and 10 mL of methanol was slowly added at 0 °C. A 2 M aqueous HCl solution was finally added before extraction with ether (3 × 100 mL). The combined organic layers were dried with brine and magnesium sulphate. After filtration the solution was evaporated under vacuo. The resulting crude oil was purified by flash chromatography on silica gel (cyclohexane:ether, 4:1) to yield 2.2 g of a colourless oil (36%, 86%ee).

Method for Calculating Heat Areas from Temperature Curves. Heat areas were calculated from the corresponding temperature curves. Initial rates were calculated between the start (9 s) and end of each reaction (117 s). Areas (A) were approximately obtained by addition of all small areas calculated between two successive plots i and $i + 1$: $A = \Sigma A_i$ where $A_i = (t_{i+1} - t_i)[(T_i + T_{i+1})/2 - 16]$, where t_i and t_{i+1} are time coordinates for plots i and $i + 1$, T_i and T_{i+1} are temperature coordinates for plots i and $i + 1$, and A_i and A_{i+1} are heat areas calculated between plots i and $i + 1$.

Screen of 96 Alcohols With *C. antarctica* Lipase Using IRT. The immobilised lipase (25 mg/well) and 4 M toluene solutions of 96 racemic mixtures of various alcohols (0.1 mL, 0.4 mmol), containing *n*-tetradecane as an internal standard (10 μL/ well), were dispensed into 96 wells of a microtitre plate. For solubility reasons, dioxane was used as a solvent in some cases. An IR camera was used to record temperature readings, and reactions were started by the addition of 8 M toluene solution of vinyl acetate (0.1 mL, 0.8 mmol). Additions were completed in less than 15 s using a multichannel pipet robot. The experiment was run for 15 min, and the temperature readings were taken every 10 s. Upon completion, reaction mixtures were quenched with methanol and analysed by GC.

Conclusions

We report here on the evaluation and development of IRT as a means to screen a potentially large number of asymmetric catalysts or substrates in a high-throughput fashion. We have shown that a differential heat output, corresponding to various mixtures of *R*- and *S*-isomers, can be achieved and have demonstrated a good linear correlation with the observed ee values with selected alcohols and amines.

However, a potential limitation and drawback of this approach was highlighted when screening alcohols in a 96-well format. It was evident that there were limitations in detection of the heat output from reactions, and a good correlation between the observed heat output and enantioselectivity could not be obtained.

It was suggested that although IRT is less accurate than other methods, and despite its limitations, it can however provide a crude but practical initial method to rapidly visualise and narrow down a selection of substrates for further evaluation. This is acceptable in an industrial scenario, when dealing with screening campaigns involving huge number of catalysts and substrates, where the speed of analysis becomes an issue. Conventionally such analyses may take several days to complete using HPLC or GC techniques, whereas using IRT would only take a few hours (if not minutes) to crudely identify a particular structural class that can be further accurately analysed giving valuable information on structure−activity relationships for lead optimisation programmes.

Acknowledgment

N.M. thanks the European Union for a Marie Curie Industry Host fellowship under the Improving Human Potential Programme.

Received for review October 8, 2001.

OP010093K