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Enzymatic resolution of a secondary amine using novel acylating reagents

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Abstract—Phenyl allylcarbonates are useful acylating agents for the enzymatic resolution of 1-methyl tetrahydroisoquinoline.
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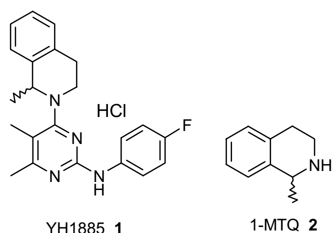
1. Introduction

As part of the development of YH1885 **1**, a potential treatment for GERD (gastro-oesophageal reflux disease) and duodenal ulcers,[1] access to both enantiomers of 1-methyl tetrahydroisoquinoline (1-MTQ) **2** was required.



Since racemic **2** was available in large quantities we decided to investigate whether an enzymatic resolution was feasible. Biocatalysis has, of course, long been recognised as an important synthetic tool in the preparation of numerous chiral intermediates[2,3] and many of these processes are now carried out on an industrial scale.[4] Lipase mediated asymmetric acylation of chiral primary amines is becoming increasingly common.[5] Surprisingly, there are very few examples of the resolution of secondary amines in the literature,[6–12] even though such building blocks are commonly used in pharmaceuticals. The resolutions that have been reported tend to suffer from poor yields or poor enantiomeric excesses.

2. Results and discussion

Initially, racemic 1-MTQ **2** was screened against a variety of common lipases and esterases using a Chiro-Kit™-TE screening kit from Altus Biologics.[13] Ethyl acetate was used as the acyl donor in diisopropyl ether. Only one hit from these 28 enzymes was obtained, that using chiroCLEC-CR, a cross-linked enzyme crystal of *Candida rugosa* lipase.[14] However, under these conditions there was only 8% of amide **3** formed after 48 h while the amine that remained was racemic. No other commercially available *Candida rugosa* lipases showed any activity (Fig. 1).

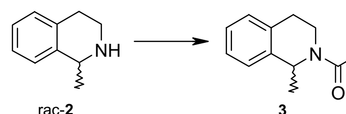


Figure 1. Reagents and conditions: *Candida rugosa* lipase, EtOAc, *i*Pr_2O, 48 h.

In the enzyme catalysed acylation of alcohols, many activated esters are used as acylating agents.[15] Enol esters are the most commonly used because of the fast rate of enzymatic acylation. The tautomerism of the enol to ketone means that the process is irreversible and product work-up is simple. However, many of these acylating agents cannot be used with amines because of high background reactions, which leads to low ee values. Several acyl donors were therefore screened in order to increase the rate and selectivity (Fig. 2).

Acyl donors **7–11** reacted with **2** under enzymatic catalysis using a large excess of the acylating reagent. There was some background reaction using **8** and **10**,

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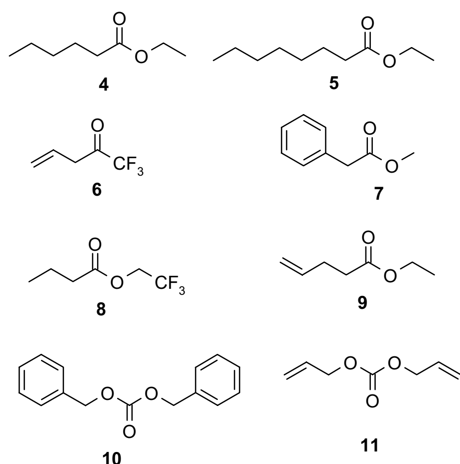

Figure 2. Acyl donors studied.

but the best results were seen using **11**. Amine **2**, which remained after 24 h, was found to be the (*S*)-enantiomer with an ee of 96%. The only drawback from this resolution was that high catalyst loading (33% by weight) was required to drive the reaction to completion. This was found to be due to the presence of allyl alcohol, which is formed as a by-product during the reaction. Doping small quantities of allyl alcohol into the reaction mixture severely retarded the rate suggesting that allyl alcohol was acting as an inhibitor. Alcohols are already known to act as inhibitors in lipase catalysed reactions.[16–19] This inhibition is dependent on the desolvation properties of the alcohol and increases as the hydrophobicity of the solvent increases.[20]

In order to overcome this problem, a series of novel allyl carbonates **12**–**21** were prepared using substituted phenols (Table 1). These were easily prepared in high yields by reacting the phenols with allyl chloroformate under phase transfer conditions. The reactivity of these carbonates can be fine tuned by varying the substituent group R on the phenol portion of the molecule. In general, the infrared C=O stretch of the carbonates correlated with its reactivity.[21] In this case, it was found that those carbonates with a C=O stretch above $1759\,cm^{-1}$ (e.g., $R = NO_2$, 2-Br, 3-Br) were too reactive, giving rise to a reaction in the absence of enzyme. Derivatives with a C=O stretch below $1759\,cm^{-1}$, notably 2-Me and 3-MeO (**18** and **20**) gave excellent selectivities and allowed the enzyme loading to be reduced to 10%. The phenol by products appeared to cause no enzyme inhibition and can be removed easily with a base wash at the end of the resolution.

Table 1. Phenylallyl carbonate acyl donors

Compound	R	ν(C=O)/cm^{-1}
12	2-NO_2	1765.2
13	2-Br	1762.6
14	3-NO_2	1762.5
15	H	1759.9
16	3-Br	1759.7
17	4-Br	1759.4
18	3-MeO	1758.8
19	4-I	1758.7
20	2-Me	1757.7
21	4-MeO	1757.5

The reaction was also enhanced by controlling the water levels because it is known that enzyme reactions in organic media require some water to maintain the conformational integrity of the enzyme.[22] In diisopropyl ether it was found that 0.25% w/w water was required to enable the reaction to proceed efficiently. One way of achieving this was to add zinc sulfate heptahydrate to the solution to act as a water buffer giving a thermodynamic water activity a_w of 0.6 (where 1 is the activity of pure water).[23] However it was noted that over time, the zinc salt caused some degradation and that this level of water reduced the selectivity of the lipase, presumably due to hydrolysis of the carbamate formed. Several other methods have been developed for controlling water levels in organic media.[24] It was more convenient to attain this water activity by using toluene containing 0.05% w/w water.

Because the amount of water present is much less (even though its activity is the same), there is much less background hydrolysis with selectivity being excellent. A solvent screen had previously shown that toluene containing no added water was less selective than diisopropyl ether. Moreover, the enzyme loading could now be reduced to 2%. To keep the water level constant over the course of the acylation, the reaction was performed in a closed system containing a reaction flask attached to another containing saturated sodium chloride solution at 50 °C. This solution had a water activity of 0.75,[25] slightly above the desired value for the acylation itself, which could possibly be due to a less than perfect equilibrium between the two flasks. This closed system reduced the reaction time from 21 to 8 h. These optimised conditions provided the (*S*)-amine with a 46% yield and an ee of 99.6% whilst the (*R*)-carbamate was recovered with a 47% yield and an ee of 98.4% (Fig. 3).

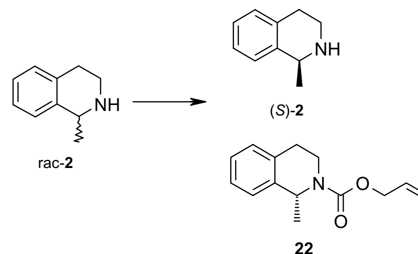

Figure 3. Reagents and conditions: **18** or **20**, *Candida rugosa* lipase, toluene, 0.05 wt% water.

The initial enzyme screen was repeated using 3-methoxyphenyl-allylcarbonate **18** as the acyl donor in toluene with 0.05% w/w water. This time every lipase tested

showed some activity. Several other commercially available *Candida rugosa* lipases were then reacted with the (*R*)-enantiomer to leave (*S*)-**2** with >99% ee and good recovery.[26] One enzyme, Subtilisin protease, showed some selectivity for the (*S*)-enantiomer under these conditions to give (*R*)-**2**.

3. Conclusions

Substituted phenyl allylcarbonates can be used to increase the rate of acylation of 1-methytetrahydroisoquinoline with lipases. It is anticipated that these phenyl allylcarbonates could be useful in other enzymatic resolutions where the reactivity and selectivity can be altered for a particular substrate by altering the substitution on the phenol.

4. Experimental

4.1. General methods

Racemic 1-methyltetrahydroisoquinoline was obtained from Yuhan Corporation. All other chemicals were obtained from commercial sources and used without further purification. NMR spectra were recorded with a Bruker 400 MHz spectrometer. HPLC analyses were performed on a Beckman Coulter 126/166 liquid chromatograph using a Phenomenex Aqua 150 × 4.6 mm 5 μ C18 column. The eluents used were 0.1% methanesulfonic acid in water and 0.1% methanesulfonic acid in acetonitrile, at 15–90% organic over 15 min then holding at 90% organic for 3 min, with solvent flow at 1 mL/min and UV detector at 210 nm. Chiral HPLC analysis was performed using a Chiralcel-OD column, isocratic analysis with 3% hexane in methanol as eluent, 1.5 mL/min solvent flow and UV detector at 220 nm. MS analyses were performed on a Waters Micromass ZQ.

4.2. Screening procedure

1-Methyltetrahydroisoquinoline (115 mg) was dissolved in diisopropyl ether (30 mL). One millilitre of this solution was added to a series of vials in a ChiroKit™-TE screening kit. Ethyl acetate (100 μL) was added to each vial. The kit was shaken in an orbital shaker at 30 °C for 48 h. A sample was taken from each vial to determine whether any reaction had taken place.

4.3. General procedure for the synthesis of carbonates 12–21

The phenol (50 mmol) and tetra-*n*-butylammonium chloride hydrate (100 mg, 0.35 mmol) were dissolved in dichloromethane (40 mL) and stirred with 4 M NaOH (20 mL) at 0–5 °C. Allyl chloroformate (6 mL, 56.5 mmol) was added slowly. After 1 h, the two layers were separated and the organic layer washed with 2 M NaOH (10 mL), then dried over $MgSO_4$ and evaporated to give the crude carbonate as an oil (88–97%). The oils were purified by distillation under reduced pressure.

4.4. Enzyme resolution using 18

1-MTQ (5 g, 34 mmol) and 3-methoxyphenyl allylcarbonate (4.65 g, 22.3 mmol) were stirred in toluene (50 mL) containing 0.05% w/w water. This can be conveniently achieved by stirring toluene and excess water together then separating off the water to leave a water–saturated toluene layer. ChiroCLEC-CR (100 mg) was added and the reaction stirred at 30 °C with the flask connected in a closed system to another flask containing a saturated sodium chloride solution at 50 °C. The reaction was followed by HPLC. After 8 h, the enzyme was filtered off and washed with toluene (10 mL). The organic layer was washed with 2 M HCl (2 × 25 mL) and the combined acid was then washed with toluene (10 mL). The pH of the aqueous layer was then adjusted to 12 with 10 M NaOH and then extracted with TBME (2 × 50 mL). The TBME was dried over $MgSO_4$ and evaporated to give (*S*)-1-methyltetrahydroisoquinoline as a colourless oil (2.3 g, 46%, 99.65 ee). ^1H NMR ($CDCl_3$): δ 1.46 (d, 3H, $J = 6.8$ Hz), 1.9 (br s, 1H), 2.73 (dt, 1H, $J = 16.3, 4.8$ Hz), 2.87 (m, 1H), 3.02 (m 1H), 3.26 (dt, 1H, $J = 12.8, 5.0$ Hz), 4.10 (q, 1H, $J = 6.8$ Hz), 7.10 (m, 4H); MS MH^+ 148.3 (100), 138.1 (20). The toluene layer was stirred with 2 M NaOH (25 mL), then dried over $MgSO_4$ and evaporated to give carbamate **22** as a colourless oil (3.7 g, 47%, 98.4% ee). ^1H NMR ($CDCl_3$): δ 1.47 (d, 3H, $J = 6.8$ Hz), 2.76 (dt, 1H, $J = 16.1, 3.8$ Hz), 2.94 (br s, 1H), 3.29 (br m, 1H), 4.16 (br m, 1H), 4.64 (dt, 2H, $J = 5.5, 1.5$ Hz), 5.22 (dq, 2H, $J = 10.5, 1.5$ Hz), 5.33 (dq, $J = 17.2, 1.5$ Hz), 5.97 (m, 1H), 7.15 (m, 4H); MS MH^+ 232.1 (95), 188.2 (100), 145.1 (75).

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