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Designing enzymatic resolution of amines

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A new strategy, utilizing IR and mass spectrometry, has been developed to design appropriate reagents and reaction conditions for enantioselective enzymatic protection of amines with readily removable protecting groups.

Stereoselective enzymatic acylation of alcohols *via* transesterification in organic solvents has become a useful strategy in enzymatic synthesis.[1] This method has been further improved to eliminate the problems of reversible reactions and product inhibition by using irreversible acyl donors,[2,3] and among these reagents enol esters have proven to be the most useful.[3] Much less developed, however, is the enzymatic protection of amines,[4] as two major problems are often encountered: (i) amines are much more nucleophilic than alcohols and react non-enzymatically with the esters commonly used in the enzymatic acylation of alcohols, and (ii) unlike esters, which can be readily cleaved under basic conditions, once amines are acylated, harsh conditions are often required to liberate the free amine.

We have systematically investigated a number of potentially useful amine protecting reagents and their reactivities and conditions in order to develop a general strategy for the selection of appropriate protecting reagents. To tackle the first problem, the reactivities of a number of amine protecting reagents were studied by comparing, with TLC, the amount of spontaneous background reaction with an amine in a non-reaction-suppressing (toluene) or reaction-suppressing solvent (3-methylpentan-3-ol),[4d,i,j] and by measuring the IR absorption maxima of the carbonyl groups [ν(C=O)]. The ν(C=O) values reflect the C=O bond length and correlate with the reactivity of carbonyl compounds; the larger the wavenumber, the shorter the C=O bond, and the more reactive the carbonyl group (Fig. 1).[5] The correlation between the IR absorption maxima and the reactivity is most accurate for comparing compounds with similar structures, but can also be useful for estimating the reactivity of dissimilar esters and carbonates.

As seen in Fig. 1, amine protecting reagents can be categorized into three categories depending on their reactivity with amines of interest: (*a*) reagents that react spontaneously with amines; (*b*) reagents that spontaneously react with amines but can be suppressed under special conditions; (*c*) reagents that do not react spontaneously with amines. Based on our experience, reagents in category (*a*) are not useful for selective enzymatic protection of amines. Reagents in category (*b*) may be useful under conditions that suppress spontaneous reactions (high dilution and/or use of a reaction-suppressing solvent such as 3-methylpentan-3-ol[4i,j]). Reagents in category (*c*) are perhaps the most useful and can be used under any condition compatible with the enzyme, so the use of high concentrations of reactants is not a problem and in many cases is actually beneficial for the reaction. Since reagents in category (*b*) do not have good leaving groups, they are poor reagents for protecting alcohols, and in fact can be used to selectively protect amines in the presence of hydroxy groups (Table 1, entry 6). In contrast, some of the reagents in categories (*a*) and (*b*) possess good leaving groups and are thus useful for the protection of alcohols and amino alcohols (*e.g.* **7**–**9**).

To solve the second problem of liberating free amines, we then selected reagents that will give readily removable amine protecting groups.[6] These exercises allowed us to quickly identify several novel (*e.g.* **6**, **8**, **11**), and some known (*e.g.* **9**,[4i] **10**[4e]) enzymatic amine protecting reagents, along with suitable reaction conditions for their use.

Benzylisopropenyl carbonate **8** is also a mildly activated reagent that gives benzyl carbamates under spontaneous reaction suppressing conditions. Dibenzyl carbonate **11** is a less reactive version of reagent **8** and useful for amine resolution when used under highly concentrated reaction conditions.

Previous attempts to use **11** in amine resolutions have been unsuccessful,[4e,7] perhaps due to poor design of reaction conditions.

Allyl pent-4-enoate **6** is a less reactive version of **9** and can be used under highly concentrated conditions whereas reagent **9** requires use of spontaneous reaction-suppressing conditions.

Representative new examples of using these protecting reagents/reaction conditions for enzymatic resolution of amines, including the pharmaceutically important 1-amino-indane,[4j] are shown in Table 1. Reagents **6**, **10** and **11** are especially useful, given that they are readily available, that the

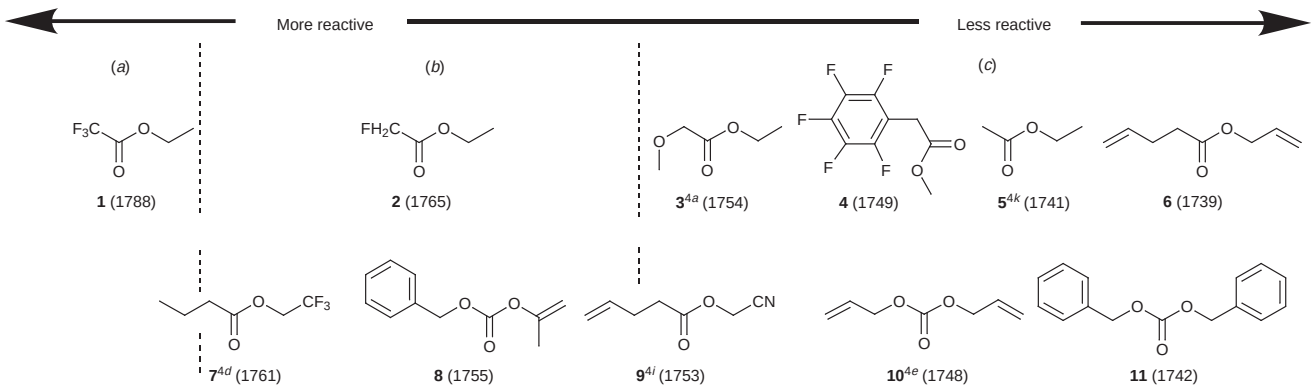
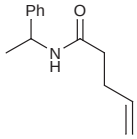
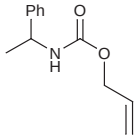
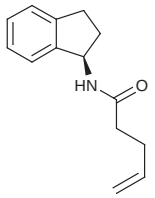
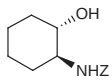
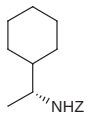
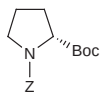


Fig. 1 Reactivity of protecting reagents and their usefulness in enzymatic protection: (*a*) too reactive, (*b*) useful under spontaneous reaction suppressing conditions and (*c*) useful under reaction promoting conditions. The number in parenthesis is IR absorption maxima for the carbonyl group.

Table 1 Examples of amine resolution using the newly designed amine protecting reagents and reaction conditions†

Entry	Product	Reagent	Conditions[a]	Yield (%)[b]	Ee (%)	$[\alpha]_D^{24}$[c]
1		**6**	A	43	(*R*) 99%	+98 (0.73)
2		**10**	A	46	(*R*) 83%	+48 (0.74)
3		**11**	A	33	(*R*) 99%	+44 (0.59)
4		**8**	B	34	(*R*) 86%	+38 (0.85)
5		**6**	A	46	(*R*) 99%	+56 (0.89)
6		**11**	A	36	(*S*,*S*) 82%	+1.8 (0.80)
7		**11**	A	41	(*R*) 81%	–2.8 (0.47)
8		**11**	A	19	(*R*) 57%	+19 (0.70)

[a] A: toluene (high concentration), *Candida antarctica* lipase (CAL); B: hexane (dilute), CAL. [a] Isolated yield. [b] In 10^{-1} deg cm^2 g^{-1}.[c] Concentration (*c*) in $CHCl_3$ shown in parenthesis.

reaction conditions are simple and user friendly (does not even require a pH meter), and that they give amides and carbamates that are widely utilized as amine protecting groups.[6]

Finally, since the efficiency of enzymatic reactions may not correlate with chemical reactivity, quantitative mass spectrometry[8] has been used to compare reagents **6** and **11** for their efficiency in *Candida antarctica* lipase catalyzed protection of amines. The amount of protected amine, formed in an enzymatic reaction containing equimolar amounts of protecting reagents **6** and **11** but with a limiting amount of amine, was measured by directly injecting a quenched reaction mixture into a mass spectrometer and comparing the peak intensities of the products to those of internal standards.‡ Compound **6** was found to be approximately five times as efficient as **11** in enzymatic amine protection. Thus, the chemical reactivity of a reagent determined by its IR absorption combined with a rapid assessment of its enzymatic reactivity using mass spectrometry illustrated in this study provides a new effective strategy for the development of new protecting reagents and conditions for efficient enzymatic amine resolutions. We have used **6** and **11** for enantioselective enzymatic transformation of more than 50 amines so far and work is in progress to further expand the scope of their application.

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Notes and references

† Condition A: Amine (0.94 mmol), toluene (230 μl), protecting reagent (1.69 mmol), molecular sieves 4 Å powder (114 mg) and *Candida antarctica* lipase (20 mg) was stirred for 48–70 h at 24 °C. The mixture was directly chromatographed (SiO_2; hexanes–Et_2O, 3:1) to give the products. The ees were determined by HPLC (Chiralpak AD or Chiralcel OD-H).

Condition B: (±)-Methylbenzylamine (168 mg, 1.39 mmol), hexane (10 ml), **8** (197 mg, 1.03 mmol), molecular sieves 4 Å powder (920 mg), and *Candida antarctica* lipase (240 mg, Sigma) was stirred for 60 h at 24 °C. The reaction was filtered through Celite, the filtrate was diluted with Et_2O, washed (dilute HCl; brine), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed (SiO_2; hexanes–Et_2O, 4:1) to give the product (120 mg, 34%).

‡ Measurement of reaction efficiency using mass spectrometry: (*R*)-α,4-Dimethylbenzylamine (35 mg, 0.29 mmol), **6** (80 mg, 0.57 mmol), **11** (138 mg, 0.57 mmol), toluene (3 ml) molecular sieves 4A (300 mg), and *Candida antarctica* lipase (20 mg) was stirred for 24 h at room temperature. After filtering through Celite, a portion of the filtrate (5 μl) was mixed with an internal standard (10 μl of a 1:1 solution of **14**:**12**), and injected directly into a PE SCIEX API100 electrospray mass spectrometer in the positive ionization mode. The relative amount of products formed was determined by the peak intensity ratio of the $[M + H^+]$ peaks (218/204 = 14.5) of the pent-4-enamides and the $[M + Na^+]$ peaks (292/278 = 3.14) of the benzyl carbamates (14.5:3.14 = 4.6:1).

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