Pergamon

Tetrahedron Letters 42 (2001) 2617–2619

TETRAHEDRON LETTERS

Measurement of enantiomeric excess of amines by mass spectrometry following kinetic resolution with solid-phase chiral acylating agents

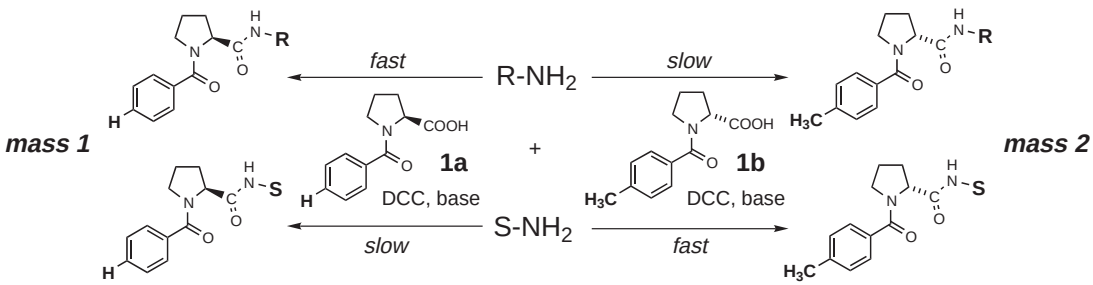
David D. Díaz, Sulan Yao and M. G. Finn*

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

Received 12 February 2001; accepted 14 February 2001

Abstract—Mass-tagged chiral activated esters on derivatized polystyrene have been used for the measurement of enantiomeric excess of amines by kinetic resolution and electrospray ionization mass spectrometry.

The discovery and refinement of enantioselective catalysts by combinatorial means is an accelerating endeavor in organic, biological, and organometallic chemistry.[1] As with all things combinatorial, analytical methods are at the heart of the enterprise, and a variety of techniques for measuring catalytic activity and/or asymmetric induction have been reported.[2] We have recently developed a method for the determination of the enantiomeric excess of alcohols and amines by kinetic resolution and subsequent mass spectrometry analysis.[3] An equimolar mixture of chiral acylating agents of opposite absolute configuration is used, which differ in a substituent remote to the chiral center. The mixture thereby comprises a 'pseudo-racemate' in which the mass of each component is correlated to its absolute configuration. The acids **1a** and **1b** shown in Scheme 1, in combination with DCC, is a simple example of such a system. Acylation of alcohols or amines is performed under parallel kinetic resolution[4] conditions, in which the 'mass tags' are used in large excess, generating ester or amide products of differing mass. The relative amounts of these products, measured by ESI-MS, give the enantiomeric composition of the starting substrate using Eq. (1) and two calibration measurements.[5] Here we describe the preparation and use of resin-based acylating agents designed to make the process more convenient.



Scheme 1.

Abbreviations: DCC, 1,3-dicyclohexylcarbodiimide; ESI-MS, electrospray ionization mass spectrometry; NMM, *N*-methylmorpholine; PyBOP, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TCT, 2,4,6-trichloro[1,3,5]triazine.

Keywords: enantiomeric resolution; enantiomeric analysis; mass spectrometry; solid phase; amines; amino esters.

* Corresponding author.

0040-4039/01/$ - see front matter
PII: S0040-4039(01)00259-3

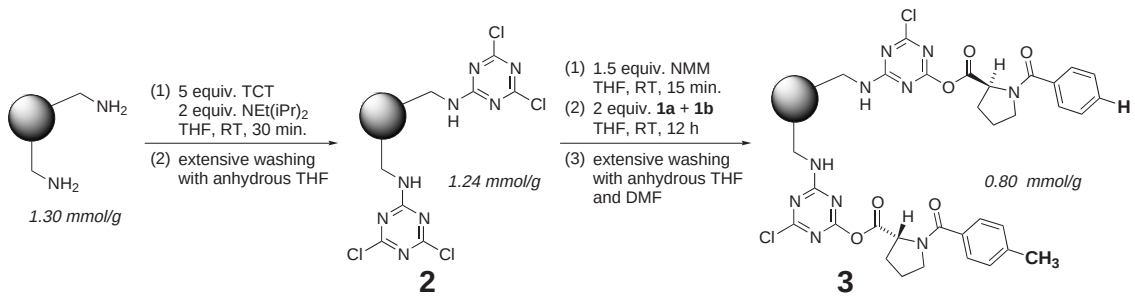
$$\%\ ee = \left(\frac{(y-1)(s+1)}{(y+1)(s-1)}\right) \quad (1)$$

where s is the ratio of fast and slow acylation rate constants (kinetic resolution selectivity factor) and y is the observed intensity ratio of the two masses of interest, corrected for the difference in ionization efficiencies (determined by analysis of racemic amine).

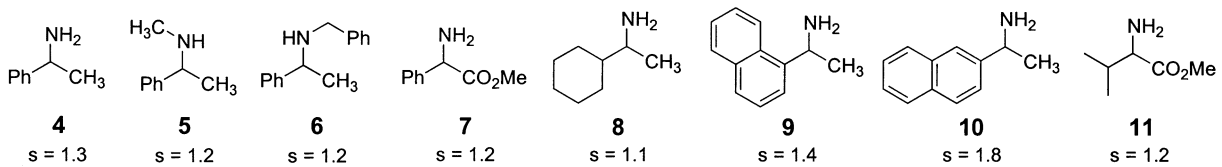
Commercially available aminomethyl-polystyrene resin was treated with 2,4,6-trichloro[1,3,5]triazine[6] in the presence of $NEt(iPr)_2$ (Scheme 2). The dichlorotriazine resin **2** was activated with *N*-methylmorpholine and condensed with an excess of an equimolar mixture of the carboxylic acids **1a** and **1b**.[3] Each reaction proceeded to completion as monitored by IR spectroscopy. Alternatively, resins were also prepared separately with **1a** and **1b**, and then combined in equimolar amounts, achieving the same results as described below.

Amines **4**–**11** were analyzed on an approximately 0.5 μmol scale using a 20-fold molar excess of **3** in the presence of a catalytic amount of DMAP.[7] After acylation, each mixture was processed by a simple procedure of filtration, evaporation, addition of methanol, and direct-injection ESI-MS in 5 μL injection aliquots, each of which contained no more than 1 nmol of the target amides. The standard practice[3] of averaging the results of three injections was followed, giving reproducible intensity ratios to standard deviations of 1–3%; [M+ Na^+] ions were dominant; a representative spectrum is shown in Fig. 1.

Fig. 1 summarizes the results for eight primary and secondary amines, available in both enantiomerically pure and racemic forms, each as samples of 20, 50, 70 and 90% *ee*.[8] In spite of the generally low kinetic resolution selectivity factors s (Eq. (1)) observed,[3] most (25 out of 32) measurements fall within 10% *ee* of the actual value. The compounds analyzed with the least accuracy were **7**, **8**, and **11**, which show the lowest s values. It should be noted that the actual acylating agent is likely to be the solution-phase *N*-acylpyridinium salt derived from the reaction of **3** with DMAP—the same as in our initial, solution-phase method. The resin-bound activated esters serve only as an accessible reservoir of chiral, mass-tagged, acyl units. The observed s-values are therefore the same in the two methods and the nature of the resin has little effect on kinetic resolution selectivity, and thus on the accuracy of the method.[9]



Scheme 2.



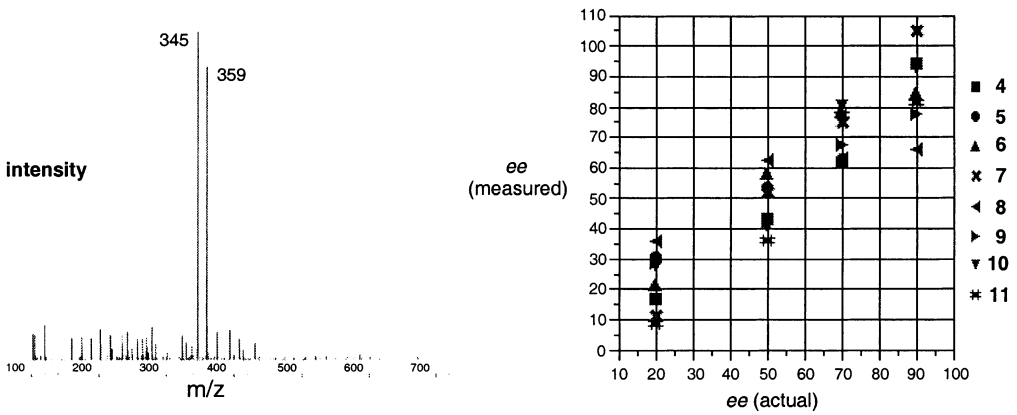


Figure 1. Top: structures and selectivity factors (s values). Bottom left: ESI-MS analysis of the products derived from *rac*-**4** and resin **3**. Bottom right: actual versus measured enantiomeric excess for amines **4**–**11**.

The principal advantages of resin **3**, making the method more easily applied to automation, are: (1) the relatively clean nature of the amides obtained, since excess acylating agent is retained on the resin and additional activating reagents (such as DCC or PyBOP) are not needed; (2) the convenience of measuring out a single free-flowing resin for each analysis; and (3) the ease of work-up. As previously observed,[3] analyses can be speeded further by combining samples containing acylated products of different molecular weight (obtained from different amines) for each ESI-MS run. The preparation of new mass-tagged reagents and their use in the screening of asymmetric catalysts will be reported in due course.

Acknowledgements

The authors would like to thank the Skaggs Institute for Chemical Biology for support of this work; S.Y. is a Skaggs Postdoctoral Fellow. D.D. thanks the Ministerio de Educacion y Cultura of Spain for an FPU fellowship. The authors are grateful to Dr. Jianhua Guo for many helpful contributions.

References

1. (a) Shimizu, K. D.; Cole, B. M.; Krueger, C. A.; Kuntz, K. W.; Snapper, M. L.; Hoveyda, A. H. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1704–1707; (b) Sigman, M. S.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1998**, *120*, 4901–4902; (c) Janes, L. E.; Löwendahl, A. C.; Kazlauskas, R. J. *Chem. Eur.* **1998**, *4*, 2324–2331; (d) Porte, A. M.; Reibenspies, J.; Burgess, K. *J. Am. Chem. Soc.* **1998**, *120*, 9180–9187; (e) Gilbertson, S. R.; Collibee, S. E.; Agarkov, A. *J. Am. Chem. Soc.* **2000**, *122*, 6522–6523; (f) Sigman, M. S.; Vachal, P.; Jacobsen, E. N. *Angew. Chem., Int. Ed.* **2000**, *39*, 1279–1281; (g) Taylor, S. J.; Morken, J. P. *J. Am. Chem. Soc.* **1999**, *121*, 12202–12203; (h) Brouwer, A. J.; Van der Linden, H. J.; Liskamp, R. M. J. *J. Org. Chem.* **2000**, *65*, 1750–1757.
2. (a) Infrared thermography: Moates, F. C.; Somani, M.; Annamalai, J.; Richardson, J. T.; Luss, D.; Wilson, R. C. *Ind. Eng. Chem. Res.* **1996**, *35*, 4801–4803; Taylor, S. J.; Morken, J. P. *Science* **1998**, *280*, 267–270; Reetz, M. T.; Becker, M. H.; Kühling, K. M.; Holzwarth, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 2647–2650; (b) Colorimetric assays: Cooper, A. C.; McAlexander, L. H.; Lee, D.-H.; Torres, M. T.; Crabtree, R. H. *J. Am. Chem. Soc.* **1998**, *120*, 9971–9972; Lavastre, O.; Morken, J. P. *Angew. Chem., Int. Ed.* **1999**, *38*, 3163–3165; (c) Circular dichroism: Ding, K.; Ishii, A.; Mikami, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 497–501; (d) Electrochemistry: Beer, G.; Niederalt, C.; Grimme, S.; Daub, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 3252–3255; (e) Mass spectrometry: Sawada, M.; Takai, Y.; Yamada, H.; Yamaoka, H.; Azuma, T.; Fujioka, T.; Kawai, Y.; Tanaka, T. *Chem. Commun.* **1998**, 1569–1570; Grigorean, G.; Ramirez, J.; Ahn, S. H.; Lebrilla, C. B. *Anal. Chem.* **2000**, *72*, 4275–4281; Reetz, M. T.; Becker, M. H.; Klein, H.-W.; Stöckigt, D. *Angew. Chem., Int. Ed.* **1999**, *38*, 1758–1761.
3. Guo, J.; Wu, J.; Siuzdak, G.; Finn, M. G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1755–1758.
4. Vedejs, E.; Chen, X. *J. Am. Chem. Soc.* **1997**, *119*, 2584–2585.
5. $I_{\text{mass 1}}/I_{\text{mass 2}} = yq$ where I is the observed mass spectrum peak intensity and q is the ionization correction factor. For a description of the calibration measurements, the derivation of Eq. (1), and a sample calculation, see Ref. 3 and its Supporting Information, or contact the authors at mgfinn@scripps.edu.
6. For other uses of TCT in solid-phase synthesis, see: (a) Attardi, M. E.; Porcu, G.; Taddei, M. *Tetrahedron Lett.* **2000**, *41*, 7391–7394; (b) Attardi, M. E.; Falchi, A.; Taddei, M. *Tetrahedron Lett.* **2000**, *41*, 7395–7399; (c) Falchi, A.; Taddei, M. *Org. Lett.* **2000**, *2*, 3429–3431; (d) Masala, S.; Taddei, M. *Org. Lett.* **1999**, *1*, 1355–1357; (e) Dulcere, J.-P.; Tawil, M.; Santelli, M. *J. Org. Chem.* **1990**, *55*, 571–575.
7. Representative procedure: resin **3** (10 mg, 8 μmol) was suspended in 300 μL THF and stirred for 5 min. DMAP (0.04 μmol) in 5 μL THF and a solution of amine (0.4 μmol) in 5 μL toluene were added. The reaction mixture was stirred or agitated at room temperature for 24 h, and then filtered. The solvent was evaporated and the residue was taken up in 2 mL MeOH and transferred to an HPLC autosampler vial for ESI-MS analysis. Details of the mass spectrometry procedures can be found in Ref. 3. NMM may be used in place of DMAP, but a stoichiometric amount of base (with respect to the amine being acylated) must be employed to obtain reliable absolute values. For the use of 4-dialkylaminopyridines as acylation catalysts, see: Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569–583.
8. Stock solutions of amines were prepared in dry toluene solvent. Solutions of different enantiomeric excess were enriched in the *R*-enantiomer for all amines except **6** and **11**, which were enriched in the *S*-enantiomer.
9. The use of a polystyrene-PEG-NH_2 resin resulted in increased acylation yield due to its more flexible nature, but no change in the observed values of s or enantiomeric excess. We were unable to convert alcohols to esters in sufficiently high yield using **3** and a variety of bases and reaction conditions.