Configurational Stability of N-Protected α-Amino Aldehydes

William D. Lubell and Henry Rapoport*

Contribution from the Department of Chemistry, University of California, Berkeley, California 94720. *Received August 18, 1986*

Abstract: A three-step synthesis of *N*-(9-(9-phenylfluorenyl))-L-alaninal (**5**) from alanine is described. Exposure to silica or nonnucleophilic base causes no detectable racemization of this α-amino aldehyde. The 9-(9-phenylfluorenyl) N-protecting group also maintains the configurational integrity of α-amino aldehyde **5** during C–C bond-forming reactions, providing enantiomerically pure products from Wittig reaction, aldol condensation, and addition of Grignard reagent.

The synthetic applications of optically active N-protected α-amino aldehydes have increased dramatically with the rising interest in the synthesis of polyfunctional amino acids,[1] peptide analogues,[2] and amino sugars.[3] In attempting to retain enantiomeric purity, many of these syntheses rely on the immediate utilization of cold, unpurified solutions of *N*-(*tert*-butoxycarbonyl)amino aldehydes,[1,2] since these intermediates are configurationally labile and racemize even upon rapid chromatography on silica gel.[4] Furthermore, evidence indicates that BOC-protected amino aldehydes may also partially racemize under the C–C bond forming reaction conditions in which they are employed.[5,6] Use of an *N*-trityl amino aldehyde with the above precautions has been observed to proceed with at least 90% ee;[6] however, the extreme ease with which the trityl group is lost under even mildly acidic conditions significantly limits its use.

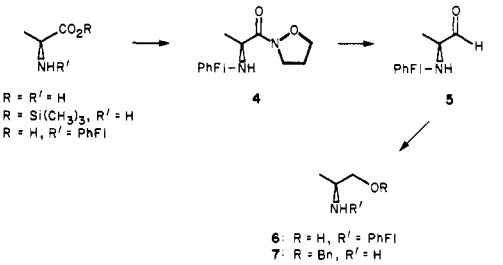
To overcome this problem of configurational instability, we have prepared *N*-(9-(9-phenylfluorenyl))-L-alaninal (**5**) from L-alanine as a white crystalline solid after silica gel purification. *N*-(Phenylfluorenyl)-L-alaninal is optically stable and can undergo nucleophilic attack without loss of enantiomeric purity. The 9-(9-phenylfluorenyl) (PhFl) protecting group, which is much more stable to solvolysis than the trityl group,[7] acts as a steric pocket that shields the α-carbon proton, preventing deprotonation and racemization of aldehyde **5** during its preparation, purification, and subsequent reactions. Deprotection can then be effected by hydrogenolysis or acid cleavage.

Results and Discussion

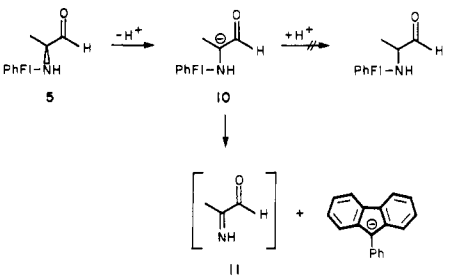
Preparation of *N*-(9-(9-phenylfluorenyl))-L-alaninal (5). Selective nitrogen protection of L-alanine (**1**) with the 9-(9-phenylfluorenyl) group was accomplished by modifying the process used for synthesis of *N*-trityl amino acids.[8] Alanine was initially protected as trimethylsilyl ester **2**, followed by alkylation of the amine with 9-bromo-9-phenylfluorene in $CHCl_3/CH_3CN$ with Et_3N as a base and $Pb(NO_3)_2$ as a halogen ion scavenger.[9] Cleavage of the weak silicon–oxygen bond occurs during acidic isolation, producing *N*-(PhFl)-L-alanine (**3**).

Analogous to observation in peptide synthesis with *N*-trityl amino acids,[10] attempts to prepare *N*-(PhFl)-L-alanine isoxazolidide (**4**) by acid chloride or mixed anhydride methods gave poor yields of amide **4**. Use of dicyclohexyl carbodiimide, however, in conjuction with hydroxybenzotriazole in THF, produced **4** in high yield. Reduction of crystalline isoxazolidide **4** proceeds through a stable metal chelated intermediate, as that observed during reduction of *N*-methoxy-*N*-methylamides.[11] This intermediate precludes overreduction, so that upon hydrolysis and silica gel purification primarily crystalline *N*-(PhFl)-L-alaninal (**5**) is obtained with only trace amounts of β amino alcohol **6**.

Scheme I. Preparation of *N*-(9-(9-Phenylfluorenyl))-L-alaninal (**5**)



Scheme II



Optical Purity Studies of 5. To test if *N*-(PhFl)-L-alaninal (**5**) racemized during its preparation and chromatographic purification on silica gel, **5** was first reduced with $NaBH_4$ to β-amino alcohol **6**. Alkylation of the potassium alkoxide of **6** with benzyl bromide and subsequent nitrogen deprotection with TFA in CH_3CN/H_2O at 80 °C for 4 h produced *O*-benzylalaninol (**7**). Parallel acylations of **7** with both L- and DL-*N*-(phenylsulfonyl)prolyl chloride and HPLC analysis of the diastereomeric amides **8** proved that *N*-(PhFl)-L-alaninal (**5**) was >99.5% enantiomerically pure.

Encouraged by this result, we conducted a study of the configurational stability of **5**, using optical rotation to assay for enantiomeric content of **5** after exposure to room temperature and refluxing solutions of THF, alone or in the presence of silica gel or triethylamine. Although the optical rotation of **5** did not decrease after exposure to these various conditions, the optical rotation assay itself seemed limited in its ability to detect small

(1) (a) Ohfune, Y.; Nishio, H. *Tetrahedron Lett.* **1984**, *25*, 4133–4136. (b) Garner, P. *Tetrahedron Lett.* **1984**, *25*, 5855–5858.
(2) (a) Rich, D. H. *J. Med. Chem.* **1985**, *28*, 263–273. (b) Hanson, G. J.; Lindberg, T. *J. Org. Chem.* **1985**, *50*, 5399–5401.
(3) Garner, P.; Ramakanth, S. *J. Org. Chem.* **1986**, *51*, 2609–2616.
(4) (a) Rittle, K. E.; Homnick, C. F.; Poticello, G. S.; Evans, B. E. *J. Org. Chem.* **1982**, *47*, 3016–3018. (b) Stanfield, C. F.; Parker, J. E.; Kanellis, P. *J. Org. Chem.* **1981**, *46*, 4797–4798.
(5) Miles, N. J.; Sammes, P. G.; Kennewell, P. D.; Westwood, R. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2299–2305.
(6) Dellaria, J. F., Jr.; Maki, R. G. *Tetrahedron Lett.* **1986**, *27*, 2337–2340.
(7) Solvolysis studies show 9-chloro-9-phenylfluorene to be 6000 times less reactive than trityl chloride. Bolton, R.; Chapman, N. B.; Shorter, J. *J. Chem. Soc.* **1964**, 1895.
(8) Barlos, K.; Papaioannou, D.; Theodoropoulos, D. *J. Org. Chem.* **1982**, *47*, 1324–1326.
(9) Christie, B. D.; Rapoport, H. *J. Org. Chem.* **1985**, *50*, 1239.
(10) (a) Velluz, L.; Amiard, G.; Bartos, J.; Goffinet, B.; Heymes, R. *Bull. Soc. Chim. Fr.* **1956**, 1464. (b) Stelakatos, G.; Theodropoulos, D. M.; Zervas, L. *J. Am. Chem. Soc.* **1959**, *81*, 2884–2887.
(11) (a) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815–3818. (b) Fehrentz, J.-A.; Castro, B. *Synthesis* **1983**, 676–678.

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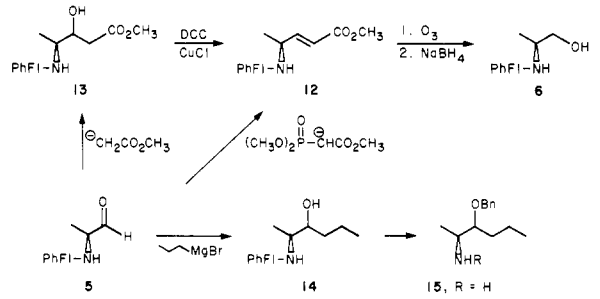
Scheme III. C–C Bond-Forming Reactions of **5**



amounts of racemization. We thus turned to a chromatographic method to scrutinize if any racemization of **5** had occurred after refluxing for 8 h in THF containing 100 mol % of triethylamine.

This severe treatment destroyed nearly 50% of **5**; however, after silica gel purification, the recovered aldehyde still possessed a high optical rotation. Conversion of aldehyde **5** to *O*-benzylalaninol (**7**) followed by coupling of **7** to both L- and DL-mandelic acid and HPLC analysis of diastereomeric amides **9** proved the recovered **5** to be of >99% enantiomeric purity (the limits of detection).

Elemental and proton NMR analysis showed the other major component from this reaction to be 9-phenylfluorene. The formation of this hydrocarbon suggests that removal of the α-carbon proton assures elimination of the stabilized aromatic 9-phenylfluorenyl anion to form imine **11** (Scheme II). Because this facile elimination occurs faster than inversion and reprotonation of sterically congested anion **10**, racemization of *N*-(PhFl) amino aldehyde **5** cannot ensue.

Choice of Chiral Auxillary for HPLC Analysis. To demonstrate the enantiomeric purity of amino ether **7** via liquid chromatography it was necessary to prepare a diastereomeric derivative. Previously excellent results had been obtained in preparing and separating diastereomeric *N*-(phenylsulfonyl)prolyl amides.[12] Amino ether **7** was similarly N-acylated with benzenesulfonylprolyl chloride, and the diastereomeric amides **8** were separable by HPLC; however, the necessary conditions were highly sensitive and difficult to reproduce. Seeking an alternative chiral auxillary we prepared mandelic amides **9**. The α-hydroxyl group of the mandelate derivative stabilized the necessary binding of amide **9** to silica gel in the absorbed state[13] and provided easy separation of the diastereomeric amides. We thus continued our HPLC studies with only mandelate derivatives.

Reactivity of 5. We next tested the reactivity and optical stability of *N*-(PhFl)amino aldehyde **5** in a Wittig reaction, aldol condensation, and addition of a Grignard reagent (Scheme III). Seeking only to demonstrate enantiomeric purity, we made no attempt to influence the diastereomeric selectivity of these reactions.

The ylide of trimethyl phosphonoacetate reacts cleanly with **5** in THF to produce only trans α,β-unsaturated methyl ester **12**. Ozonolysis of **12** followed by reduction with $NaBH_4$ resulted in β-amino alcohol **6**, along with "abnormal" ozonolysis product 9-(ethylamino)-9-phenylfluorene which arises from a Baeyer–Villiger type reaction that may proceed through an α-amine oxide intermediate.[14] Conversion of **6** to mandelic amide **9** and HPLC analysis proved **6** to be >99% optically pure.

Addition of the lithium enolate of methyl acetate to **5** in THF produced a 1/1 mixture of γ-amino β-hydroxy methyl esters **13**. Elimination to the α,β-unsaturated ester **12** was accomplished by using cuprous chloride catalyzed addition of dicyclohexyl carbodiimide to the hydroxyl group of **13** to provide the *O*-alkylisourea followed by heating to eliminate dicyclohexyl urea.[15] Analysis of **12** as described above again proved it to be of >99% enantiomeric purity.

Addition of excess *n*-propyl Grignard reagent to **5** in THF produced a 3/1 mixture of diastereomeric amino alcohols **14**, which were separated by chromatography. Benzylation of the potassium alkoxide of the major diastereomer and subsequent nitrogen deprotection with TFA in CH_3CN/H_2O gave (2*S*)-2-amino-3-(benzyloxy)hexane (**15**). Acylation of amino ether **15** with the mixed anhydride from isobutyl chloroformate and L- and DL-mandelic acid followed by HPLC analysis of the resulting diastereomeric amides **16** demonstrated amino either **15** to be >99% enantiomerically pure.

Conclusion

We have demonstrated both the configurational stability and synthetic utility of *N*-(PhFl)-L-alaninal (**5**). The 9-(9-phenylfluorenyl) N-protecting group prevents enolization of an α-amino aldehyde by obstructing removal of the α-carbon proton. Thus after refluxing *N*-(PhFl)amino aldehyde **5** in THF with silica gel or triethylamine, no detectable racemization is observed, while C–C bond forming reactions with **5** proceed to provide products >99% optically pure as established by HPLC analysis. Considering the wide variety of available optically pure amino acids and the compatibility of this process with other functionality, this method for preparing optically stable α-amino aldehydes exhibits considerable potential for chirospecific synthesis.

Experimental Section

General. Tetrahydrofuran (THF) was distilled from $LiAlH_4$; methylene chloride and chloroform were distilled from P_2O_5; acetonitrile and diisopropylamine were distilled from CaH_2. Final reaction mixture solutions were dried over Na_2SO_4. Melting points were determined on a Swissco melting point apparatus and are uncorrected. ^1H NMR spectra were recorded in $CDCl_3$ and are reported in ppm (δ units) downfield of internal tetramethylsilane ($(CH_3)_4Si$).

***N*-(9-(9-Phenylfluorenyl))-L-alanine (3).** To a stirred suspension of L-alanine (18 g, 0.2 mol) in 0.6 L of $CHCl_3/CH_3CN$ (5/1) in a Morton flask was added $(CH_3)_3SiCl$ (26 mL, 0.2 mol) at room temperature under a nitrogen atmosphere. This mixture was heated under reflux for 2 h and then allowed to cool to room temperature. Addition of Et_3N (56 mL, .4 mol) at a rate sufficient to maintain gentle reflux was followed by the addition of $Pb(NO_3)_2$ (40 g, 0.12 mol) and a solution of 9-bromo-9-phenylfluorene (64.2 g, 0.2 mol) in $CHCl_3$ (0.2 L). The resulting mixture was vigorously stirred for 48 h at room temperature when excess MeOH (0.5 mol) was added. Filtration followed by evaporation gave a residue which was partitioned between Et_2O (1 L) and precooled 5% aqueous citric acid (1 L). The organic phase was washed with 1 N NaOH (2 × 0.4 L) and H_2O (2 × 0.2 L), and the combined aqueous layers were washed with Et_2O (0.4 L), cooled to 0 °C, and neutralized with glacial AcOH. The precipitated product was extracted into Et_2O (4 × 0.3 L), and the combined organic layers were washed with water (0.2 L) and dried. Evaporation left **3** as a light yellow foam (55 g, 84%) which was used without further purification. Careful crystallization of **3** from EtOAc/hexane produced a white solid: mp 158–161 °C; TLC (3/1, EtOAc/hexane, aluminum backed silica) R_f 0.25; $[\alpha]^{20}_D$ 163° (*c* 1.0, $CHCl_3$); ^1H NMR δ 0.72 (d, 3 H, J = 6.9), 2.58 (m, 1 H), 7.36 (m, 11 H), 7.71 (m, 2 H). Anal. Calcd for $C_{22}H_{19}NO_2$: C, 80.2; H, 5.8; N, 4.2. Found: C, 80.4; H, 5.6; N, 4.2.

***N*-(9-(9-Phenylfluorenyl))-L-alanine Isoxazolidide (4).** Isoxazolidine hydrochloride[16] (1.7 g, 15.5 mmol) was dissolved in 26 mL of THF/H_2O (25/1), and the solution was stirred for 0.5 h at room temperature with anhydrous K_2CO_3 (6 g, 43.4 mmol) and then transferred by cannula away from the precipitate into a 0 °C solution of *N*-(PhFl)-L-alanine (**3**, 4 g, 12.2 mmol) in THF (80 mL). Dicyclohexylcarbodiimide (3.14 g, 15 mmol) and hydroxybenzotriazole hydrate (2 g, 15 mmol) were added, and the vessel was flushed with nitrogen and stirred at 0 °C for 18 h. Concentration in a rotary evaporator in the presence of silica gel (2 g) gave a powdery solid which was placed on a column of silica gel (230–400 mesh, 300 g). Chromatography, eluting with hexane/ethyl acetate (2/1), and evaporation of the collected fractions left a white solid which was recrystallized from ethyl acetate/hexane to provide 4.1 g (87%) of

(12) (a) Knudsen, C. G.; Rapoport, H. *J. Org. Chem.* **1983**, *48*, 2260–2266. (b) Maurer, P. J.; Takahata, H.; Rapoport, H. *J. Am. Chem. Soc.* **1984**, *106*, 1095–1098.

(13) (a) Helmchen, G.; Nill, G.; Flockerzi, D.; Schüle, W.; Youssef, M. S. K. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 62–63. (b) Helmchen, G.; Völter, H.; Schüle, W. *Tetrahedron Lett.* **1977**, 1417–1420.

(14) (a) Young, W. G.; McKinnis, A. C.; Webb, I. D.; Roberts, J. D. *J. Am. Chem. Soc.* **1946**, *68*, 293–296. (b) Story, P. R.; Burgess, J. R. *Tetrahedron Lett.* **1968**, 1287–1292. (c) Ramachandran, V.; Murray, R. W. *J. Am. Chem. Soc.* **1978**, *100*, 2197–2201.

(15) Miller, M. J. *J. Org. Chem.* **1980**, *45*, 3131–3132.

(16) Cupps, T. L.; Boutin, R. H.; Rapoport, H. *J. Org. Chem.* **1985**, *50*, 3972–79.

isoxazolidide **4**: mp 188–189 °C; TLC (3/1 EtOAc/hexane) R_f 0.4; $[\alpha]^{20}_D$ −242° (*c* 2.0, $CHCl_3$); ^1H NMR δ 1.13 (d, 3 H, *J* = 7.0), 1.94 (m, 2 H), 2.56 (m, 1 H), 3.07 (m, 2 H), 3.54 (m, 2 H), 7.29 (m, 11 H), 7.64 (m, 2 H). Anal. Calcd for $C_{25}H_{24}N_2O_2$: C, 78.1; H, 6.3; N, 7.3. Found: C, 77.7; H, 6.2; N, 7.1.

***N*-(9-(9-Phenylfluorenyl))-L-alaninal (5).** Lithium aluminum hydride (0.15 g, 4 mmol) was added to a stirred, 0 °C solution of isoxazolidide **4** (1.5 g, 3.9 mmol) in THF (40 mL). Reduction was complete within 15–20 min. The mixture was hydrolyzed with $KHSO_4$ (0.85 g, 6.25 mmol) in H_2O (20 mL). Ethyl acetate (50 mL) was then added, and the aqueous phase was further extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (40 mL), dried, and evaporated to an oil which was chromatographed on silica gel (150 g, 230–400 mesh), eluting with hexane/EtOAc (9/1). Evaporation left **5** as a white crystalline solid (1.1 g, 90%): mp 105–107 °C; $[\alpha]^{20}_D$ −20° (*c* 5.2, $CHCl_3$); TLC (3/1 hexane/EtOAc) R_f 0.58; ^1H NMR δ 0.98 (d, 3 H, *J* = 7.2), 2.64 (q, 1 H), 7.33 (m, 11 H), 7.68 (m, 2 H), 9.23 (d, 1 H, *J* = 1.7). Anal. Calcd for $C_{22}H_{19}NO$: C, 84.3; H, 6.1; N, 4.5. Found: C, 84.2; H, 6.2; N, 4.3.

***N*-(9-(9-Phenylfluorenyl))-L-alaninol (6).** A solution of aldehyde **5** (0.1 g, 0.32 mmol) in a 1/1 mixture of isopropyl alcohol/THF (4 mL) was cooled to 0 °C and treated with a precooled suspension of $NaBH_4$ (0.038 g, 1 mmol) in isopropyl alcohol (1 mL). The solution was stirred for 1.2 h at 0 °C and then poured into rapidly stirred NaH_2PO_4 (1.0 M, 5 mL) and extracted thoroughly with ethyl acetate (2 × 10 mL) and chloroform (2 × 10 mL). The combined organic layers were dried and concentrated to a residue which was chromatographed on silica gel (Chromatotron, eluting with 1/1 ethyl acetate/hexane). Evaporation left **6** as a crystalline solid (80 mg, 70%): mp 138–140 °C; TLC (3/1, hexane/EtOAc) R_f 0.18; $[\alpha]^{20}_D$ 281° (*c* 1.0, $CHCl_3$); ^1H NMR δ 0.73 (d, 3 H, *J* = 6.6), 2.32 (m, 2 H), 3.02 (d, 2 H, *J* = 5.0), 7.32 (m, 11 H), 7.72 (m, 2 H). Anal. Calcd for $C_{22}H_{21}NO$: C, 83.8; H, 6.7; N, 4.4. Found: C, 84.0; H, 6.6; N, 4.3.

***O*-Benzyl-L-alaninol (7).** To a stirred suspension of washed potassium hydride (0.25 g, 5.25 mmol) in THF (40 mL) at room temperature under a nitrogen atmosphere was added dropwise a solution of *N*-(PhFl)-L-alaninol (**6**, 1.39 g, 4.4 mmol) in THF (10 mL). This solution was stirred for 1 h, then treated with benzyl bromide (0.75 mL, 6.25 mmol), and after 15 min poured into a vigorously stirred solution of 1 M NaH_2PO_4 (30 mL). The aqueous layer was washed with EtOAc, and the combined organic layer was dried, filtered, evaporated, and chromatographed on silica gel (30 g, 230–400 mesh) with 9/1 hexane/EtOAc. The fractions were evaporated, leaving 1.5 g of a clear oil: TLC (3/1, hexane/EtOAc) R_f 0.58; $[\alpha]^{20}_D$ −88° (*c* 1.0, $CHCl_3$); ^1H NMR δ 0.68 (d, 3 H, *J* = 6.5), 2.46 (m, 2 H), 3.05 (m, 2 H), 4.23 (s, 2 H), 7.3 (m, 11 H), 7.6 (m, 2 H). Anal. Calcd for $C_{29}H_{27}NO$: C, 85.9; H, 6.7; N, 3.4. Found: C, 86.1; H, 6.8; N, 3.4.

This oil was dissolved in a 1/1/0.1 solution of TFA/CH_3CN/H_2O (42 mL), heated at 80 °C for 4 h, then cooled to 0 °C, basified to pH 10 (10% K_2CO_3), and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was then extracted with 1 M H_3PO_4 (2 × 30 mL), and the aqueous layers were combined and basified to pH 10 (3 N NaOH). the basic solution was extracted with CH_2Cl_2 (3 × 30 mL), and the combined organic layers were dried and evaporated to leave **7** as an oil: 0.5 g (69%); ^1H NMR δ 7.26 (m, 5 H), 4.45 (s, 2 H), 3.31 (m, 1 H), 3.1 (m, 2 H), 1.4 (s, 2 H), 0.96 (d, 3 H, *J* = 6).

For further characterization **7** was converted to its hydrochloride with methanolic HCl: TLC (3/1, CH_3CN/H_2O) R_f 0.7; mp 146–148 °C; $[\alpha]^{20}_D$ 4° (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{10}H_{16}NOCl$: C, 59.5; H, 8.0; N, 6.9. Found: C, 59.4; H, 8.1; N, 6.8.

***Methyl (4S)-4-((9-(9-Phenylfluorenyl))amino)-2-pentenoate* (12).** A solution of trimethyl phosphonoacetate (0.48 mL, 3 mmol) in THF (10 mL) was cooled to 0° C and treated with NaH (100 mg, 2 mmol, 50% dispersion in oil). The solution soon became gelatinous and was allowed to warm to room temperature and stirred 0.5 h at which time it was recooled to 0°C and treated with a solution of *N*-(PhFl)-L-alaninal (**5**, 313 mg, 1 mmol) in THF (6 mL). After 5 min at 0 °C the solution was warmed to room temperature, stirred an additional 15 min, and treated with H_2O (25 mL). After being stirred for 10 min, the mixture was partitioned between ethyl acetate (30 mL) and brine (30 mL). The organic layer was dried, filtered, and evaporated, resulting in an oil that was chromatographed on 100 g of silica gel (230–400 mesh) with 10% EtOAc in hexane for elution. Evaporation yielded 330 mg (90%) of **12** as a white solid: mp 104–106 °C; TLC (3/1, hexane/EtOAc) R_f 0.48; $[\alpha]^{20}_D$ −57° (*c* 1.0, $CHCl_3$); ^1H NMR δ 0.97 (d, 3 h, *J*. = 6.7), 2.84 (m, 1 H, *J* = 6.7), 3.61 (s, 3 H), 5.22 (dd, 1 H, *J* = 15.54, 0.8), 6.45 (dd, 1 H, *J* = 15.54, 7.5), 7.09–7.71 (br m, 13 H). Anal. Calcd for $C_{25}H_{23}NO_2$: C, 81.3; H, 6.3; N, 3.8. Found: C, 80.9; H, 6.1; N, 3.7.

Ozonolysis of 12 to *N*-(PhFl)-L-alaninol (6). A stream of ozone gas was vigorously bubbled through a −78 °C solution of **12** (0.5 g, 1.35 mmol) in MeOH (20 mL) until the solution appeared bright blue. Oxygen was then bubbled through the solution until it became visibly clear and $NaBH_4$ (300 mg) was added followed by stirring for 15 min at −78 °C, warming to room temperature, and stirring for another 1 h. The solution was then treated with 1 M NaH_2PO_4 (0.5 mL), partitioned between brine (20 mL) and EtOAc (25 mL), dried, and evaporated. The solid was redissolved in EtOAc and chromatographed on silica gel (100 g, 230–400 mesh), eluting with 3/1 hexane/EtOAc. Evaporation of the collected fractions gave two products. Eluting first was 9-(ethylamino)-9-phenylfluorene (150 mg, 53%): ^1H NMR δ 0.9 (t, 3 H, *J* = 7), 1.75 (br s, 1 H), 2.17 (q, 2 H, *J* = 7), 7.08–7.65 (br m, 13 H). Eluting second was **6** (200 mg, 47%).

***Methyl (3RS,4S)-3-Hydroxy-4-((9-(9-phenylfluorenyl))amino)pentanoate* (13).** A solution of diisopropylamine (0.17 mL, 1.2 mmol) in THF (5 mL) at −78 °C was treated with *n*-butyllithium (0.8 mL, 1.2 mmol, in hexane) and stirred 15 min whereupon methyl acetate (0.1 mL, 1.2 mmol, distilled from P_2O_5) was added. The mixture was stirred 30 min at −78 °C and a solution of *N*-(PhFl)-L-alaninal (**5**, 0.35 g, 1.1 mmol) in THF (5 mL) was added over 3 min. After 35 min MeOH (1 mL) was added and the solution was allowed to reach room temperature and then washed with 1 M NaH_2PO_4 (5 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL), and the combined organic layer was washed with brine (2 × 10 mL), dried, and evaporated to a clear oil which was chromatographed (Chromatotron eluting with 25% EtOAc in hexanes). Concentration of the collected fractions left 400 mg (94%) of the diastereomers **13** as a clear oil: TLC (1/1, hexane/EtOAc) R_f 0.46; ^1H NMR δ 0.64 (d, 3 H), 2.25 (m, 3 H), 3.58 (m, 1 H), 3.6 (s, 3 H), 7.2–7.43 (m, 11 H), 7.67 (m, 2 H). Anal. Calcd for $C_{25}H_{25}NO_3$: C, 77.5; H, 6.5; N, 3.6. Found: C, 77.3; H, 6.8; N, 3.5.

Dehydration of β-Hydroxy Ester 13 to 12. To a room temperature solution of dicyclohexylcarbodiimide (3.3 mmol, 680 mg) and cuprous chloride (0.9 mmol, 90 mg) in THF (10 mL) was added a solution of β-hydroxy ester **13** (2.2 mmol, 870 mg) in THF (15 mL), and the resulting mixture was warmed to 55 °C and stirred for 18 h. The mixture was allowed to cool to room temperature and diluted with EtOAc (30 mL). Evaporation in the presence of 1 g of silica gel gave a powdery solid which was chromatographed on a column of silica gel (150 g), eluting with EtOAc/hexane (1/9). Concentration of the collected fractions left **12** as a clear oil (950 mg) contaminated with dicyclohexylurea. The mixture was ozonized without further purification.

***(2S,3RS)*-3-Hydroxy-2-((9-(9-phenylfluorenyl))amino)hexane (14).** *N*-(PhFl)-L-alaninal (**5**, 1.82 g, 5.8 mmol) was dissolved in THF (70 mL), cooled to −40 °C, and treated with a freshly prepared tetrahydrofuran solution of *n*-propylmagnesium bromide (1 N, 17 mmol). The solution was allowed to warm to room temperature over 1.25 h and then it was poured into 1 M NaH_2PO_4 (50 mL), and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layer was washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL) and dried. Evaporation left the diastereomeric mixture **14** as a yellow oil which was chromatographed with a gradient of ether in hexane. First fraction (0.45 g, 22%): TLC (1/1, Et_2O/hexane) R_f 0.44; ^1H NMR δ 0.55 (d, 3 H, *J* = 6.4), 0.82 (t, 3 H, *J* = 7), 1.2 (br m, 4 H), 2.0 (m, 1 H), 3.05 (m, 1 H), 7.2–7.4 (m, 11 H), 7.7 (m, 2 H). Second fraction (1.4 g, 67%): R_f 0.32; ^1H NMR δ 0.68 (m, 6 H), 0.97–1.3 (br m, 4 H), 2.12 (m, 1 H), 2.93 (m, 1 H), 7.2–7.43 (m, 11 H), 7.7 (m, 2 H). Anal. Calcd for $C_{25}H_{27}NO$: C, 84.0; H, 7.6; N, 3.9. Found: C, 84.3; H, 7.8; N, 3.6.

(2S)-2-Amino-3-(benzyloxy)hexane (15). A solution of the major diastereomer of **14** (896 mg, 2.4 mmol) in THF (25 mL) was added dropwise to a suspension of washed potassium hydride (130 mg, 3.25 mmol) in THF (1 mL). The mixture was stirred at room temperature for 1.5 h when it was cooled to 0 °C, benzyl bromide (0.33 mL, 2.75 mmol) was added, the suspension was allowed to attain room temperature and stirred an additional 30 min, and then the mixture was poured into a chilled solution of 1 M KH_2PO_4 (25 mL). The mixture was extracted with EtOAc (3 × 25 mL), and the organic layers were combined, washed with brine (30 mL), dried, and evaporated to an oil which was chromatographed on silica gel (50 g) with 5% EtOAc in hexane for elution. Evaporation left 1.1 g of the PhFl derivative of **15** as an oil: TLC (3/1, hexane/EtOAc) R_f 0.6; ^1H NMR δ 0.61 (d, 3 H, *J* = 6.6), 0.7 (t, 3 H, *J* = 7), 1.1 (br m, 2 H), 1.52 (m, 2 H), 2.35 (m, 2 H), 3.05 (m, 1 H), 4.41 (d, 1 H, *J* = 11.9), 4.5 (d, 1 H, *J* = 11.9), 7.1–7.43 (m, 16 H), 7.7 (m, 2 H). Anal. Calcd for $C_{32}H_{33}NO$: C, 85.9; H, 7.4; N, 3.1. Found: C, 86.2; H, 7.4; N, 2.9.

To remove the PhFl group, the oil was redissolved in (1/1/0.1) TFA/CH_3CN/H_2O (21 mL) and heated at 80 °C for 8 h, after which it was cooled to room temperature. Evaporation was followed by partition between 1 N HCl (30 mL) and Et_2O (30 mL). The aqueous layer was extracted with Et_2O (25 mL), chilled to 0 °C, and then basified to pH 10 (50% NaOH). The basic solution was extracted with Et_2O (3 × 50 mL), and the combined organic layer was evaporated to leave **15** as

a clear oil: 160 mg, 32%; ^{1}H NMR δ 0.92 (t, 3 H, J = 6.9), 1.06 (d, 3 H, J = 6.6), 1.4 (m, 2 H), 1.55 (m, 4 H), 3.15 (m, 1 H), 3.23 (m, 1 H), 4.56 (m, 2 H), 7.3 (m, 5 H).

Determination of Optical Purity. Each amino ether was N-acylated with either *N*-(phenylsulfonyl)prolyl chloride[12] or mandelic acid–isobutylcarbonic acid anhydride. When racemic N-acylating agents were used, the resulting diastereomeric amides were shown to be separable and of equal concentration by analytical HPLC. When optically pure N-acylating agents were used, HPLC showed that >99% (the limit of detection) of one diastereomer was present. The *N*-(phenylsulfonyl)-proline derivatives were chromatographed on a normal phase column, eluting with CH_2Cl_2/isopropyl alcohol, 99.75/0.25. The mandelic acid derivatives were chromatographed on a reverse phase column, eluting with H_2O/CH_3CN, 75/25, 65/35, or 45/55.

Preparation of Mandelic Acid Amides. L- or D,L-mandelic acid (0.68 mmol, 103 mg) was dissolved in THF (4 mL), cooled to −15 °C, and treated with *N*-methylmorpholine (0.68 mmol, 75 μL) followed by isobutyl chloroformate (0.62 mmol, 80 μL). The resulting solution was stirred for 60 s and then a precooled solution of amino ether (0.22 mmol) in THF (1 mL) was added. The solution was stirred an additional 45 min at −15 °C. Solvent was evaporated, and the residue was partitioned between 10% aqueous citric acid (10 mL) and EtOAc (10 mL). The aqueous layer was reextracted with EtOAc (2 × 10 mL), and the combined organic layer was washed with saturated $NaHCO_3$ (2 × 10 mL) and brine (10 mL), dried, and evaporated. The residue contains the isobutyl carbonate of the mandelic amide which is suitable for HPLC analysis. More sensitive analyses result from the mandelic amide, obtained by dissolving the carbonate in MeOH (5 mL) and treating with pH 8 phosphate buffer (5 mL) for 17 h.

Configurational Stability Studies of *N*-(9-(9-Phenylfluorenyl))-L-alaninal (5). Solutions of 0.1 M *N*-(PhFl)-L-alaninal (**5**) in THF alone or in the presence of 100 mol % of triethylamine or 100 wt % silica gel were stirred for 24 h at room temperature or 1 h at reflux and then evaporated. The optical rotations of the remaining solids showed no decrease in rotation. An analogous solution of **5** and triethylamine in THF was heated at reflux 8 h, and an aliquot was concentrated to an oil which was chromatographed (Chromatotron), eluting with 10% EtOAc in hexane. Eluting first was 9-phenylfluorene followed by aldehyde **5** which showed no loss in optical rotation. The remaining solution was chilled to 0 °C, treated with excess $NaBH_4$, stirred 1 h, and poured into 1 M NaH_2PO_4. The aqueous layer was extracted with EtOAc, and the combined organic layer was washed with brine, dried, filtered, and evaporated to an oil which was chromatographed on silica gel, eluting with hexane/EtOAc, 2/1. Concentration of the collected fractions gave **6** which was subsequently converted to its mandelic amide and shown to be >99% enantiomerically pure by HPLC analysis.

Simple General Acid–Base Catalysis and Virtual Transition States for Acetylcholinesterase-Catalyzed Hydrolysis of Phenyl Esters

Scott A. Acheson, Dimitra Dedopoulou, and Daniel M. Quinn*

Contribution from the Department of Chemistry, University of Iowa, Iowa City, Iowa 52242.
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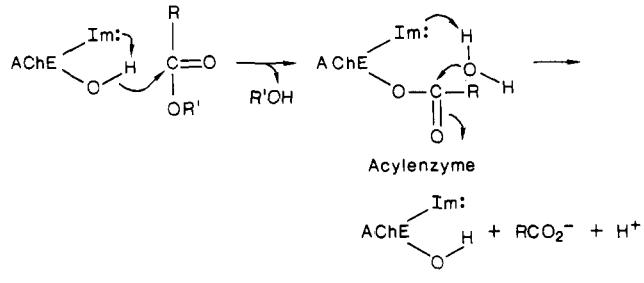
Abstract: Acetylcholinesterase-catalyzed hydrolyses of the acetyl esters phenyl acetate and *o*-nitrophenyl acetate and of the chloroacetyl esters phenyl chloroacetate and *p*-methoxyphenyl chloroacetate have been investigated. V's are quantitatively similar for the constituents of each pair of esters, which indicates that deacylation is partly rate limiting when the enzyme is saturated by substrate. Solvent deuterium isotope effects for V/K are near unity, which is consistent with virtual acylation transition states that are prominently rate limited by nonchemical events [Quinn, D. M.; Swanson, M. L. *J. Am. Chem. Soc.* **1984**, *106*, 1883–1884]. On the other hand, solvent deuterium isotope effects for V fall in the range 1.6–2.26 and are interpreted in terms of transition states that are stabilized by solvation catalytic proton bridges. The Eyring plot constructed from initial velocities of AChE-catalyzed hydrolysis of *o*-nitrophenyl acetate at $[S]_o \gg K$ is nonlinear downward and is interpreted in terms of prominent rate determination from both acylation and deacylation. However, the solvent isotope effect for the reaction is independent of temperature, which indicates that the solvent isotope effects for the acylation and deacylation components of V must be of comparable magnitude. Proton inventory plots of partial solvent isotope effects on initial velocities at $[S]_o \gg K$ vs. the atom fraction of deuterium in mixed H_2O–D_2O buffers are linear for the substrates phenyl chloroacetate and *o*-nitrophenyl acetate. Therefore, AChE behaves as a simple general acid–base catalyst for the studied ester hydrolyses. pL–rate profiles (L = H, D) for hydrolysis of *o*-nitrophenyl acetate are sigmoidal in shape, and nonlinear-least-squares analysis gives $pK_a^{H_2O} = 6.31 \pm 0.03$, $pK_a^{D_2O} = 6.81 \pm 0.03$, and $^{D_2O}V_{i,lim} = 1.82 \pm 0.02$. The β-deuterium secondary isotope effect for *o*-nitrophenyl acetate hydrolysis is $^{D3}V_i = 0.96_0 \pm 0.01_7$. These results are interpreted in terms of a virtual transition state for AChE-catalyzed ester hydrolysis that is a weighted average of acylation and deacylation transition states that are each stabilized by single-proton transfers.

The broad strokes of acetylcholinesterase (AChE[1]) catalysis have been appreciated for some years.[2,3] The AChE mechansim involves nucleophilic serine and general acid–base[3,4] catalytic elements and an acylenzyme intermediate, as outlined in Scheme I, where Im represents the imidazole side chain of the active site histidine. The mechanism of Scheme I is analogous to that of the serine proteases.[5,6] The details of AChE catalysis are not well-defined, however. Rosenberry has measured kinetic solvent deuterium isotope effects of 2–3 for V of AChE-catalyzed hydrolysis of acetate esters.[3,4] Do isotope effects of this magnitude arise because the enzyme stabilizes chemical transition states via single-proton bridges (as Scheme I implies) or via concerted proton transfers (as in the case of serine protease catalyzed hydrolysis of peptide substrates[7-11])? Are proton transfers and nucleophilic

Scheme I



* Author to whom correspondence should be addressed.

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