Synthesis of (S)-2-Amino-3-(3-*tert*-butyl-5-oxo-2*H*-isoxazol-4-yl)propionic Acid

Mohamed Atfani and William D. Lubell*

Département de chimie, Université de Montréal, C. P. 6128, Succ. A, Montréal, Québec, Canada H3C 3J7

Received January 18, 1995[⊗]

An efficient method for synthesizing enantiopure isoxazole amino acids has been developed. (2*S*)-2-Amino-3-(3-tert-*tert*-butyl-5-oxo-2*H*-isoxazol-4-yl)propionic acid (**1**) was synthesized in three steps from L-γ-methyl *N*-(9-(9-phenylfluorenyl))glutamate (**2**) in 46% overall yield. Acylation of the dianion of **2** with pivaloyl chloride provides β-keto ester **3** in 75% yield after chromatography. (2*S*)-2-[*N*-(9-(9-phenylfluorenyl))amino]-3-(3-*tert*-butyl-5-oxo-2*H*-isoxazol-4-yl)propionic acid (**4**) was isolated as a white crystalline solid from the reaction of **3** with hydroxylamine under basic conditions followed by exposure to HCl. The crystal structure of **4** confirmed the presence of a 3-*tert*-butyl-4-isoxazol-5(2*H*)-one. Title compound **1** was obtained by removal of the PhFl protecting group of **4** using either trifluoroacetic acid in CH_2Cl_2 or lithium in liquid ammonia. Alkylation of **4** with iodomethane provided a mixture containing (2*S*,4*S*)-methyl 2-[*N*-(9-(9-phenylfluorenyl))amino]-3-(3-*tert*-butyl-4-methyl-5-oxo-4*H*-isoxazol-4-yl)propionate (**5**) and (2*S*)-methyl 2-[*N*-(9-(9-phenylfluorenyl))amino]-3-(2-methyl-3-*tert*-butyl-5-oxo-2*H*-isoxazol-4-yl)propionate (**6**). Single crystal X-ray analyses of the structures of **4**–**6** provide insight into the mechanism of alkylation as well as the configurational stability of *N*-(9-(9-phenylfluorenyl))amino carbonyl compounds.

Introduction

Glutamate mediates synaptic excitation of several dissimilar receptor subtypes in the central nervous system.[1] Pharmacological classification of these subtypes has been best accomplished through the use of excitatory amino acids with glutamate-like structures that serve as selective agonists.[2] Activation of glutamate receptors has been associated with memory and learning processes,[3] and excessive activation of glutamate receptors has been implicated in neuronal degradation and neuropathological conditions such as Alzheimer's disease.[4] Synthesis of glutamate receptor ligands has thus attracted considerable interest in the development of both memory enhancing and neuroprotective therapeutics. The powerful insecticidal activity of excitatory amino acids has also generated interest in their synthesis for use as potential pesticides.[5]

Several potent and selective glutamate receptor ligands possess isoxazole heterocycles attached by flexible and rigid carbon-chain linkers to α-amino acid portions (Figure 1).[2,6–10] For example, α-amino-3-hydroxy-5-alkyl-4-isoxazolealkanonic acids have been used extensively to define and study excitatory amino acid receptors.[2,6,7] Isolation and synthesis of the antifungal antibiotic TAN-950 A has led to the preparations of 3-alkyl-4-isoxazol-5(2*H*)-one amino acids that also exhibit high affinity for glutamate receptors.[8] Resolution of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and analogues has demonstrated that the neuroexcitatory activity of 3-hydroxy-4-isoxazole amino acids is related to the stereochemistry of the α-carbon.[9] Similarly, α-carbon stereochemistry is important to the pharmacological activity of 3-alkyl-4-isoxazol-5(2*H*)-one amino acids.[8d] In addition, synthetic modifications have shown that neuroexcitatory activity is contingent on the isoxazole sub-

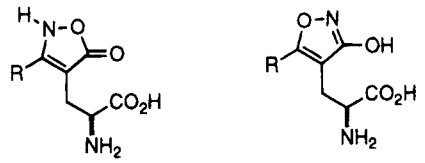


Figure 1. Isoxazole amino acid analogues.

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1995.

(1) (a) Nakanishi, S. *Science* **1992**, *258*, 597 and references cited therein. (b) Hollmann, M.; Heinemann, S. *Annu. Rev. Neurosci.* **1994**, *17*, 31.

(2) (a) *Excitatory Amino Acid Related Agents. Bioorg., Med. Chem. Lett. Symposia in Print.* Johnson, G., Ed. **1993**, *3*, 9. (b) Johnson, G.; Bigge, C. F. *Annu. Rep. Med. Chem.* **1991**, *26*, 11. (c) Hansen, J. J.; Krogsgaard-Larsen, P. *Med. Res. Rev.* **1990**, *10*, 55. (d) Watkins, J. C.; Krogsgaard-Larsen, P.; Honoré, T. *Trends Pharm. Sci.* **1990**, *11*, 25.

(3) (a) Collingridge, G. L.; Singer, W. *Trends Pharm. Sci.* **1990**, *11*, 290. (b) Madison, D. V.; Malenka, R. C.; Nicoll, R. A. *Annu. Rev. Neurosci.* **1991**, *14*, 379.

(4) (a) Greenamyre, J. T.; Maragos, W. F.; Albin, R. L.; Penney, J. B.; Young, A. B. *Neuro-Psychopharmacol. Biol. Psych.* **1988**, *12*, 421. (b) Glutamate's role in the mechanism of long-term depression is reviewed in the following: Ito, M. *Annu. Rev. Neurosci.* **1989**, *12*, 85.

(5) Maeda, M.; Kodama, T.; Saito, M.; Tanaka, T.; Yoshizumi, H.; Nomoto, K.; Fujita, T. *Pestic. Biochem. Physiol.* **1987**, *28*, 85.

(6) Syntheses of AMPA are reported in the following: (a) Hansen, J. J.; Krogsgaard-Larsen, P. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1826. (b) Honoré, T.; Lauridsen, J. *Acta Chem. Scand.* **1980**, *B34*, 235. (c) Begtrup, M.; Sløk, F. A. *Synthesis* **1993**, 861. (d) Pevarello, P.; Amici, R.; Colombo, M.; Varasi, M. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2151.

(7) Synthesis of ATPA is reported in the following: (a) Lauridsen, J.; Honoré, T.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1985**, *28*, 668. (b) Brehm, L.; Johansen, J. S.; Krogsgaard-Larsen, P. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2059.

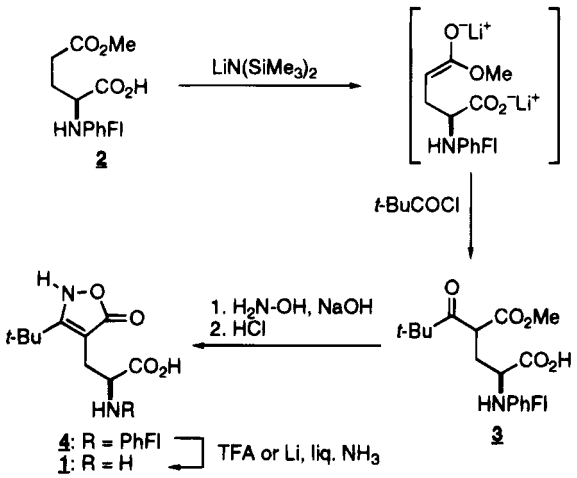
(8) Isolation and synthesis of TAN-950 A and derivatives are reported in the following: (a) Tsubotani, S.; Funabashi, Y.; Takamoto, M.; Hakoda, S.; Harada, S. *Tetrahedron* **1991**, *47*, 8079. (b) Iwama, T.; Nagai, Y.; Harada, S.; Itoh, K.; Nagaoka, A. *Eur. J. Pharmocol.* **1990**, *183*, 471. (c) Iwama, T.; Nagai, Y.; Tamura, N.; Harada, S.; Nagaoka, A. *Eur. J. Pharmocol.* **1991**, *197*, 187. (d) Tamura, N.; Matsushita, Y.; Iwama, T.; Harada, S.; Kishimoto, S.; Itoh, K. *Chem. Pharm. Bull.* **1991**, *39*, 1199.

(9) Resolution of 3-hydroxy-5-alkyl-4-isoxazole amino acids are reported in the following: (a) Ebert, B.; Lenz, S.; Brehm, L.; Bregnedal, P.; Hansen, J. J.; Frederiksen, K.; Bøgesø, K. P.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1994**, *37*, 878. (b) Nielsen, B. Fisker, H.; Ebert, B.; Madsen, U.; Curtis, D. R.; Krogsgaard-Larsen, P.; Hansen, J. J. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 107. (c) Hansen, J. J.; Nielsen, B.; Krogsgaard-Larsen, P.; Brehm, L.; Nielsen, E. Ø.; Curtis, D. R. *J. Med. Chem.* **1989**, *32*, 2254. (d) Hansen, J. J.; Lauridsen, J.; Nielsen, E.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1983**, *26*, 901.

(10) Christensen, I. T.; Ebert, B.; Madsen, U.; Nielsen, B.; Brehm, L.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1992**, *35*, 3512.

0022-3263/95/1960-3184$09.00/0

Scheme 1. Synthesis of Isoxazol-5-one Amino Acid 1.



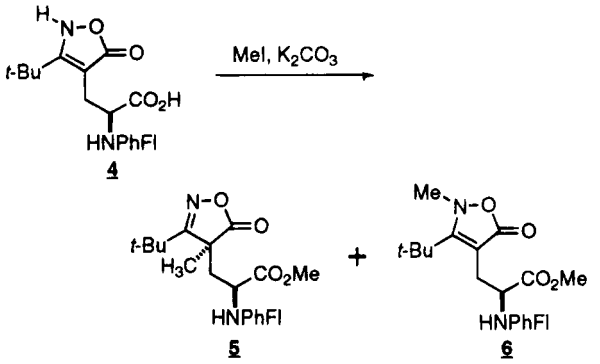
stituent and the side-chain length between the heterocycle and α-amino acid portions.[8d,10]

In light of the significance of the α-carbon stereochemistry, the isoxazole substituent, and the side-chain length to their biological activity, we have investigated the annulation of the isoxazole directly onto an enantiopure α-amino dicarboxylate in order to control all of these features during the synthesis of these glutamate receptor ligands.[11] The 5-*tert*-butylisoxazole agonist ATPA exhibits high potency and the ability to penetrate the blood–brain barrier by virtue of its lipophilicity (Figure 1).[7] Since ATPA has yet to be resolved and its 3-alkyl-4-isoxazol-5(2*H*)-one isomer **1** had not been previously synthesized, the *tert*-butylisoxazole amino acids were selected as targets for developing this strategy. We previously reported a general method for synthesizing β-keto esters via the acylation of the lithium enolate of α-*tert*-butyl γ-methyl *N*-(9-(9-phenylfluorenyl))glutamate.[12a] Glutamate-derived β-keto esters possessing primary, secondary, and tertiary alkyl as well as aromatic substituents can be prepared by our method.[12a] Toward the preparation of *tert*-butylisoxazole amino acids, we have also synthesized β-keto ester **3** in good yield via the acylation of the dianion of L-γ-methyl *N*-(9-(9-phenylfluorenyl))glutamate (**2**) with pivaloyl chloride (Scheme 1, PhFl = 9-(9-phenylfluorenyl)).[11,12b] With β-keto ester **3** in hand, we considered that (2*S*)-2-amino-3-(3-*tert*-butyl-5-oxo-2*H*-isoxazol-4-yl)propionic acid (**1**) and ATPA could both be synthesized via condition control in the addition of hydroxylamine to β-keto ester **3** and subsequent deprotection. Our strategy has now led to the first total synthesis of (2*S*)-**1** in three steps and 46% yield from glutamate **2**.[11]

Results and Discussion

The addition of hydroxylamine to ordinary β-keto esters under basic conditions followed by acidification has been extensively used to synthesize both 3-hydroxyisoxazoles and isoxazol-5(2*H*)-ones.[13–17] The mechanism of isoxazole formation has also been effectively studied using ^{13}C NMR spectroscopy to identify intermediates

Scheme 2. Alkylation of Isoxazol-5-one *N*-(9-(9-Phenylfluorenyl))amino Acid 4.



and products.[15,16] Alkyl substituents on the β-keto ester can influence the regioselectivity of isoxazole formation.[15] In addition, 3-hydroxyisoxazoles can be the favored products from the addition of hydroxylamine to the β-keto ester when the reaction is conducted at pH ≈ 10 followed by quenching the basic solution rapidly with concentrated HCl.[17]

In the case of glutamate-derived β-keto ester **3**, we found that the reaction with hydroxylamine using the pH ≈ 10 conditions gave only recovered starting material; however, a new product was produced in 69% yield as a white crystalline solid when the reaction was run at pH > 12 for 100 min followed by acidification with concentrated HCl at −5 °C. Proton NMR of the solid showed a *tert*-butyl singlet at 1.07 ppm and three aliphatic protons exhibiting geminal and vicinal coupling constants of 15.9, 9.3, and 3.5 Hz. Carbon NMR showed the disappearance of the ketone resonance at 210 ppm and the appearance of a new resonance at 90 ppm indicative of an isoxazole.[15,16] Although the spectra suggested that the heterocycle had been formed, the regiochemistry of the isoxazole remained ambiguous and an X-ray investigation of the crystalline material was performed to establish whether (2*S*)-2-[*N*-(9-(9-phenylfluorenyl))amino]-3-(3-*tert*-butyl-5-oxo-2*H*-isoxazol-4-yl)propionate (**4**) or its 3-hydroxy-5-*tert*-butyl-4-isoxazole isomer was prepared.[18]

Suitable orthorhombic crystals were grown from methanol. The image obtained from X-ray crystallographic analysis is presented in Figure 2a. This structure not only reveals that 2-[*N*-(9-(9-phenylfluorenyl))amino]-3-(3-*tert*-butyl-5-oxo-2*H*-isoxazol-4-yl)propionic acid (**4**) was in fact prepared in the reaction of hydroxylamine with **3** under the described conditions but, it also shows that **4**

(11) Atfani, M.; Lubell, W. D. Presented in part at the 8th European Conference on Organic Chemistry, Barcelona, Spain, Aug 29–Sept 3, 1993.

(12) (a) Ibrahim, H. H.; Lubell, W. D. *J. Org. Chem.* **1993**, *58*, 6438. (b) Beausoleil, E.; L'Archeveque, B.; Belec, L.; Atfani, M.; Lubell, W. D. Manuscript in preparation.

(13) Representative syntheses of isoxazl-5-ones are reported in the following: (a) Jacquier, R.; Petrus, C.; Petrus, F.; Verducci, J. *Bull. Soc. Chim. Fr.* **1970**, 2685. (b) De Sarlo, F. *Tetrahedron* **1967**, *23*, 831. (c) Boulton, A. J.; Katritzky, A. R. *Tetrahedron* **1961**, *12*, 41. (d) Katritzky, A. R.; Øksne, S.; Boulton, A. J. *Tetrahedron* **1962**, *18*, 777. (e) Uhlenhuth, R. *Liebigs Ann.* **1897**, *296*, 33. (f) Pinner, A. *Ber.* **1891**, *24*, 495 and references cited therein.

(14) Representative syntheses of 3-hydroxyisoxazoles are reported in the following: (a) Ruhland, B.; Leclerc, G. *J. Heterocycl. Chem.* **1989**, *26*, 469. (b) Jacquier, R.; Petrus, C.; Petrus, F.; Verducci, J. *Bull. Soc. Chim. Fr.* **1970**, 1978 and references cited therein.

(15) Katritzky, A. R.; Barczynski, P.; Ostercamp, D. L.; Yousaf, T. I. *J. Org. Chem.* **1986**, *51*, 4037.

(16) Auzou, G.; Rips, R.; Likforman, J.; Hazebroucq, G.; Aji, M. *Org. Magn. Reson.* **1979**, *12*, 59.

(17) Jacobsen, N.; Kolind-Andersen, H.; Christensen, J. *Can. J. Chem.* **1984**, *62*, 1940.

(18) The structures of **4**, **5**, and **6** were solved at l'Université de Montréal X-ray facility using direct methods (SHELXS 86). The author has deposited the atomic coordinates for the structures of **4**, **5**, and **6** with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

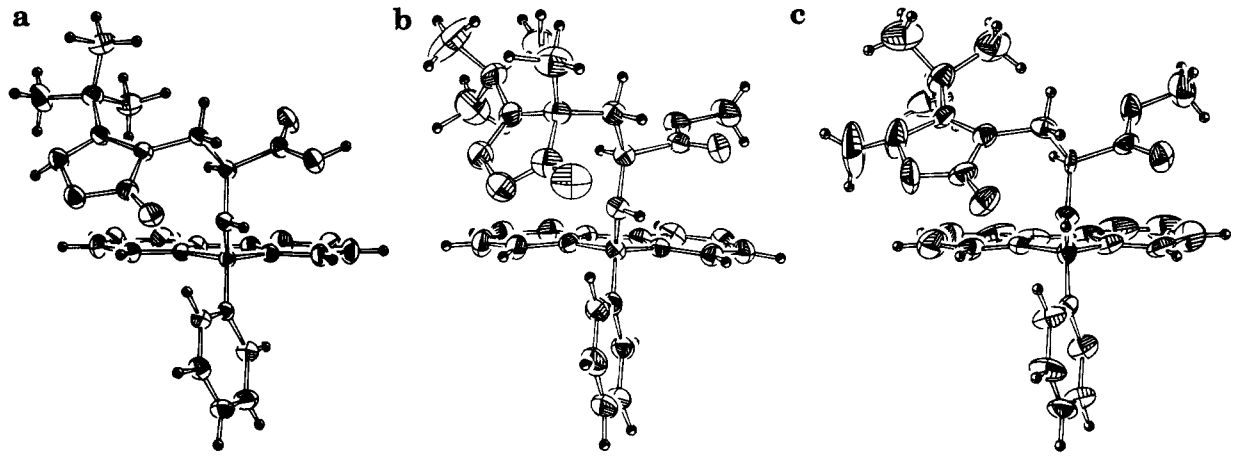


Figure 2. (a) ORTEP drawing of amino acid **4**. (b) ORTEP drawing of amino ester **5**. (c) ORTEP drawing of amino ester **6**.

exists as the 3,4-dialkylisoxazol-5(2*H*)-one tautomer.[19] Intermolecular hydrogen bonding between the acid and the carbonyl of the isoxazol-5(2*H*)-one in the crystal of **4** may contribute to the stability of this enamide tautomer.[19]

Deprotection of **4** by treatment with aqueous hydrobromic acid[7a] at 100 °C removed the PhFl group, cleaved the heterocycle with decarboxylation, and provided an imine that was isolated as 5-*tert*-butyl-Δ^5-dehydroproline methyl ester hydrobromide in 70% yield after esterification.[20] Lower temperatures and different acids gave the desired (*S*)-2-amino-3-(3-*tert*-butyl-5-oxo-2*H*-isoxazol-4-yl)propionic acid (**1**) sometimes contaminated with imine. Removal of the PhFl group was cleanly accomplished with trifluoroacetic acid in dichloromethane at a reflux for 17 h and furnished **1** in 90% yield. We also found that lithium in liquid ammonia removed the PhFl group without isoxazole ring cleavage providing **1** in 75% yield after purification on an ion-exchange resin.[21]

Since the spectral properties of isoxazol-5(2*H*)-one and 3-hydroxyisoxazole amino acids have not been studied under similar conditions, we compared **1** and authentic ATPA using ^{1}H and ^{13}C NMR in order to examine the characteristics that discriminate isoxazole amino acids.[22] The proton NMR spectra of isoxazol-5(2*H*)-one **1** and ATPA are very similar in CD_3OD acidified with trifluoroacetic acid. The α-proton of **1** is observed ~0.1 ppm downfield of the α-proton in ATPA. The feature that best distinguished amino acid **1** from ATPA was the ^{13}C NMR signal of C-4 which appeared upfield in the spectrum of **1** (δ = 91 ppm) relative to the C-4 signal of ATPA (δ = 99 ppm) in CD_3OD acidified with trifluoroacetic acid.

Alkylation of **4** was also examined in order to study the reactivity of isoxazol-5(2*H*)-one amino acids. A recent report had shown that alkylation of ordinary 4-substituted isoxazol-5(2*H*)-ones with alkyl halides provides both *C*- and *N*-alkylation products,[23] instead of the usual *N*- and *O*-alkylated products that are obtained from reactions with diazomethane[13b] and palladium-catalyzed allylation.[24] Treatment of **4** with iodomethane and potassium carbonate in DMF gave both *C*- and *N*-alkylation products, esters **5** and **6**. The chemical shift for C-4 (49 ppm) of ester **5** indicated that *C*-methylation had occurred.[16] Furthermore, the NMR spectrum indicated that a single diastereomer of **5** was produced from stereospecific *C*-alkylation. The *N*-methylated structure of ester **6** was assigned based on the *N*-CH_3 (δ = 3.3 and 42 ppm) and the C-4 (δ = 102 ppm) resonances.[16]

Single-crystal X-ray analyses confirmed the structural assignments of esters **5** and **6**, as well as established that (2*S*,4*S*)-diastereomer **5** had been prepared (Figure 2b,c).[18] The isoxazolone N−C signal bond distances are respectively 1.37 and 1.38 Å for isoxazol-5(2*H*)-one **4** and *N*-methylisoxazolone **6**, and the N−C double bond distance for *C*-methylated product **5** is 1.28 Å. The isoxazolone double bond distances between C-3 and C-4 are respectively 1.37 and 1.34 Å for **4** and **6**, and the single bond distance between C-3 and C-4 is 1.50 Å in **5**.

A model to account for the diastereospecific *C*-alkylation may be proposed on examination of the X-ray data. The structures of both starting material **4** and alkylated products **5** and **6** all adopt similar conformations in the crystalline state. Because this conformation avoids steric interactions between the *tert*-butyl and the phenylfluorenyl groups, the isoxazole *N*-(9-(9-phenylfluorenyl))-amino acids may prefer this conformation in solution. Electrophilic attack on isoxazol-5(2*H*)-one **4** in this conformation proceeds on the face that is not blocked by the phenylfluorenyl group and thereby provides (2*S*,4*S*)-diastereomer **5**. In addition, it should be noted that the crystal structures of **4**−**6** all exhibit conformations in which the α-proton and the α-acid (α-ester) carbonyl are nearly coplanar. Since α-deprotonation from the coplanar geometry is stereoelectronically less favored than from an orthogonal geometry, this arrangement of the α-proton and α-carbonyl group may contribute to the

(19) The tautomerism of isoxazl-5-ones and 3-hydroxyisoxazoles is reviewed in Elguero, J.; Marzin, C.; Katritzky, A. R.; Linda, P. *Adv. Heterocycl. Chem.* **1976**, Suppl. 1, 300.

(20) (2*S*)-5-*tert*-Butyl-Δ^5-dehydroproline methyl ester was obtained by heating a solution of *N*-(9-(9-phenylfluorenyl))amino acid **4** in 48% HBr at 100 °C for 15 min, adding MeOH, and letting the solution cool to room temperature. Removal of the hydrocarbon with hexane and EtOAc and evaporation left a red oil that crystallized from THF: mp >260 °C; ^{1}H NMR (CD_3OD acidified with CF_3CO_2H) δ 1.39 (s, 9 H), 2.38 (m, 1 H), 2.67 (m, 1 H), 3.35 (t, 2 H, J = 8), 3.84 (s, 3 H), 5.18 (dd, 1 H, J = 5.6, 10.2); ^{13}C NMR (CD_3OD acidified with CF_3CO_2H) δ 25.3, 25.4, 27.7, 36.3, 38.8, 53.9, 68.4, 170.2, 208.9; IR (KBr) 3400−2900, 2300, 1750, 1650, 1600, 1400, 1250.

(21) Lubell, W. D.; Jamison, T. F.; Rapoport, H. *J. Org. Chem.* **1990**, *55*, 3511.

(22) ATPA exhibited the following spectral properties: ^{1}H NMR (CD_3OD acidified with CF_3CO_2H) δ 1.35 (s, 9 H), 2.98 (dd, 1 H, J = 7.6, 15.3), 3.17 (dd, 1 H, J = 6.6, 15.3), 4.16 (t, 1 H, J = 7, 7.2); ^{13}C NMR (CD_3OD acidified with CF_3CO_2H) δ 24.6, 29, 35.4, 53.6, 99.3, 170.9, 171.3, 177.7; ATPA·HBr, FT-IR (KBr) 3426, 3274, 2965, 2532, 1639, 1593, 1409.

(23) Batra, S.; Seth, M.; Bhaduri, A. P. *J. Chem. Res. (S)* **1992**, 139.

(24) Moreno-Mañas, M.; Pérez, M.; Pleixats, R. *Tetrahedron* **1994**, *50*, 515.

configurational stability of α-*N*-(phenylfluorenyl)amino carbonyl compounds.[25]

Conclusion

We have developed a strategy to prepare isoxazole α-amino acids via the annulation of the heterocycle onto an *N*-protected amino dicarboxylate. The utility of this method was demonstrated by the synthesis of (*S*)-2-amino-3-(3-*tert*-butyl-5-oxo-2*H*−isoxazol-4-yl)propionic acid (**1**) in three steps and 46% overall yield from γ-methyl *N*-(9-(9-phenylfluorenyl))glutamate. A detailed characterization of the isoxazole amino acid products has been accomplished by ^{1}H and ^{13}C NMR as well as by crystal structure analyses of *N*-(9-(9-phenylfluorenyl))amino derivatives **4**−**6**. Work is in progress to extend this methodology in order to synthesize ATPA as well as a variety of heterocyclic amino acids using alternative conditions and different α-amino dicarboxylates.[26]

Experimental Section

General. Unless otherwise noted, all reactions were run under nitrogen atmosphere and distilled solvents were transferred by syringe. THF and ether were distilled from sodium/benzophenone immediately before use; 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and CH_2Cl_2 were distilled from CaH_2. DMF was distilled from 4 Å molecular sieves. Final reaction mixture solutions were dried over Na_2SO_4. Chromatography was on 230−400 mesh silica gel; TLC on aluminum-backed silica plates. Mass spectral data, HRMS (EI), were obtained by the Université de Montréal Mass Spectroscopy facility. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in $CDCl_3$. Chemical shifts are reported in ppm (δ units) downfield of internal TMS, and coupling constants are given in hertz. Chemical shifts for aromatic PhFl carbons are not reported.

(2*S*,3*RS*)-6,6-Dimethyl-5-oxo-4-(methyloxycarbonyl)-2-[*N*-(9-(9-phenylfluorenyl))amino]heptanoate (3). A −10 °C solution of HMDS (10.5 mL, 50 mmol) in 10 mL of THF was treated with *n*-butyllithium (15 mL of a 2.5 M solution in hexane, 37.5 mmol), stirred for 30 min, cooled to −78 °C, and treated with a solution of γ-methyl *N*-(9-(9-phenylfluorenyl))-L-glutamate (**2**, 5 g, 12.5 mmol)[12] in THF (15 mL). The reaction mixture was stirred at −78 °C for 1.5 h, treated with trimethylacetyl chloride (4.4 mL, 37.5 mmol) in THF (3 mL), stirred an additional 45 min, and poured into 1 M NaH_2PO_4 (50 mL). The mixture was extracted with EtOAc (4 × 25 mL), and the combined organic phases were washed with cold water (3 × 10 mL) and brine (2 × 15 mL), dried, filtered, and evaporated to a residue. The residue was dissolved in ether from which trimethylacetate crystallized and was removed by filtration. The residue was purified by chromatography on silica gel with a gradient of 17−60% EtOAc in hexane as eluant and gave 4.56 g (75%) of a 1.5:1 mixture of diastereomers. **3**: ^{1}H NMR δ 1.07 (s, 9 H), 1.22 (s, 9 H), 1.55 (m, 2 H), 2.03 (m, 1 H), 2.25 (m, 1 H), 2.5 (m, 2 H), 3.47 (s, 3 H), 3.7 (s, 3 H), 3.87 (dd, 1 H, *J* = 2.8, 8.8), 4.37 (dd, 1 H, *J* = 2.6, 10), 7.1−7.6 (m, 26 H); ^{13}C NMR δ 25.9, 26, 33, 33.6, 45.3, 45.6, 48, 49.7, 52.1, 52.3, 53.9, 54.6, 72.6, 72.7, 170.1, 170.2, 178.2, 178.3, 210, 210.2; HRMS calcd for $C_{30}H_{32}NO_5$ (MH^+) 486.2280, found 486.2250.

(*S*)-2-[*N*-(9-(9-Phenylfluorenyl))amino]-3-(3-*tert*-butyl-5-oxo-2*H*-isoxazol-4-yl)propionic acid (4). A solution of hydroxylamine hydrochloride (120 mg, 1.7 mmol, 125 mol %) in 0.2 mL of H_2O was treated with 2 N NaOH (2.9 mL, 400 mol %) at 0 to −5 °C and stirred vigorously for 15 min. A solution of β-keto ester **2** (670 mg, 1.4 mmol, 100 mol %)[12] in 4 mL of methanol was added dropwise to the chilled mixture over 100 min with continued stirring. The mixture was then treated with 3.2 mL of 6 N HCl, let sit at 0 °C for 24 h, and concentrated on a rotary evaporator to an aqueous solution that was extracted with EtOAc (3 × 25 mL). The organic phases were combined and evaporated to a residue that was triturated with Et_2O, leaving a white solid that recrystallized from methanol and furnished 450 mg (69%) of **4**: mp 208−210 °C; $[\alpha]^{20}_D$ −86° (*c* 0.2, MeOH); ^{1}H NMR (CD_3OD) δ 1.07 (s, 9 H), 2.74 (dd, 1 H, *J* = 3.5, 15.9), 2.9 (dd, 1 H, *J* = 9.3, 15.9), 3.05 (dd, 1 H, *J* = 3.5, 9.3), 7.29 −7.93 (m, 13H); ^{13}C NMR (CD_3OD) δ 25.5, 28.1, 34.2, 60.4, 76.4, 90.3, 169.8, 171.6, 178.1; FT-IR (KBr) 3451, 3181, 1714, 1665, 1579; UV 194, 248, 338 nm in 2.5 × 10^{-3} M MeOH; HRMS calcd for $C_{29}H_{29}N_2O_4$ (MH^+) 469.2127, found 469.2110.

(*S*)-2-Amino-3-(3-*tert*-butyl-5-oxo-2*H*-isoxazol-4-yl)propionic Acid (1). Deprotection of 4 with Li in Liquid NH_3. A solution of *N*-(9-(9-phenylfluorenyl))amino acid **4** (260 mg, 0.56 mmol) in 5 mL of THF was added to a −78 °C blue solution of lithium (78 mg, 0.011 gmol atoms) in ammonia (25 mL, distilled from Li). The mixture was brought to −50 °C, held at a reflux for 4 min, cooled to −78 °C, treated with 0.2 mL of H_2O, and let warm to rt. After the volatiles had evaporated, the residue was dissolved in 10 mL of H_2O and washed with Et_2O (3 × 20 mL). The pH was adjusted to 5.5 with 0.1 M HCl, and the aqueous was evaporated to a solid that was purified on an Amberlite IRA-400 C.P. anion exchange resin (hydroxide form, 20−50 mesh) with 95:5 H_2O: acetic acid as eluant. Evaporation of the ninhydrin positive fractions gave 95 mg (75%) of **1**: mp 105 °C; $[\alpha]^{20}_D$ 13° (*c* 0.2, MeOH); ^{1}H NMR (CD_3OD acidified with CF_3CO_2H) δ 1.36 (s, 9 H), 2.95 (dd, 1 H, *J* = 8, 15.7), 3.1 (dd, 1 H, *J* = 5.1, 15.7), 4.26 (dd, 1 H, *J* = 5.1, 8); ^{13}C NMR (CD_3OD acidified with CF_3CO_2H) δ 25.4, 28.6, 34.6, 53.6, 90.6, 170.8, 172.2, 176.1; FT-IR (KBr) 3430, 2977, 1673, 1522, 1205; HRMS calcd for $C_{10}H_{17}N_2O_4$ (MH^+) 229.1188, found 229.1200.

Deprotection of 4 with CF_3CO_2H. *N*-(9-(9-phenylfluorenyl))amino acid **4** (130 mg, 0.28 mmol) was dissolved in 25 mL of CH_2Cl_2, treated with 2 mL of CF_3CO_2H, and heated at a reflux for 17 h. The volatiles were evaporated, and the residue was treated with 10 mL of H_2O and filtered. The aqueous solution was washed with hexanes (2 × 20 mL) and then evaporated to a solid that crystallized from MeOH providing 56 mg (88%) of **1** possessing the same physical and spectral characteristics as above.

Alkylation of *N*-(9-(9-phenylfluorenyl))amino Acid 4 with Iodomethane. A solution of *N*-(9-(9-phenylfluorenyl))-amino acid **4** (670 mg, 1.43 mmol), iodomethane (0.18 mL, 2.86 mmol), and K_2CO_3 (0.4 g, 2.86 mmol) in 10 mL of DMF was stirred for 36 h. The salts were filtered and washed with EtOAc (25 mL). The combined organic phases were washed with 1 N HCl (5 × 5 mL), dried, and evaporated to a residue that was chromatographed using a gradient of 10−50% ether in hexane as eluant. First to elute was 56 mg of a product exhibiting NMR resonances that suggested a dimer.[7b] Next to elute was ester **5** (370 mg, 52%). Last to elute was ester **6** (240 mg, 34%). Esters **5** and **6** were recrystallized from ether/petroleum ether. **(2*S*,4*S*)-Methyl 2-[*N*-(9-(9-phenylfluorenyl))amino]-3-(3-*tert*-butyl-4-methyl-5-oxo-4*H*-isoxazol-4-yl)propionate (5)**: R_f = 0.35 (Et_2O/hexane, 2/1); mp 185 −187 °C; $[\alpha]^{20}_D$ −270.9° (*c* 0.34, $CHCl_3$); ^{1}H NMR δ 1.04 (s, 9 H), 1.45 (s, 3 H), 1.9 (d, 1 H, *J* = 14.8), 2.2 (m, 1 H), 2.64 (m, 1 H), 3.07 (s, 3 H), 7 −7.7 (m, 13 H); ^{13}C NMR δ 24.7, 29, 35.9, 41.5, 49.6, 51.5, 54.7, 72.8, 174.4, 175.5, 181.9; FT-IR ($CHCl_3$): 3000, 1786, 1735, 1602, 1449; HRMS calcd for $C_{31}H_{33}N_2O_4$ (MH^+) 497.2440, found 497.2439. Anal. Calcd for $C_{31}H_{32}N_2O_4$: C, 75.4; H, 6.6; N, 5.6. Found: C, 75.4; H, 6.6; N, 5.6. **(2*S*)-Methyl 2-[*N*-(9-(9-phenylfluorenyl))amino]-3-(2-methyl-3-*tert*-butyl-5-oxo-2*H*-isoxazol-4-yl)propionate (6)**: R_f = 0.12 (Et_2O/hexane, 2/1); mp 164−166 °C; $[\alpha]^{20}_D$ −176.7° (*c* 0.36, MeOH); ^{1}H NMR δ 1.11 (s, 9 H), 2.45 (dd, 1H, *J* = 4.6, 13.7), 2.6 (dd, 1 H, *J* = 9.2, 13.7), 2.68 (dd, 1 H, *J* = 4.6, 9.2), 3.27 (s, 3 H), 3.3 (s, 3 H), 7.1−7.7 (m, 13 H); ^{13}C NMR δ 28.8, 29.2, 33.7, 42.3, 51.4, 56.7, 72.8, 101.7, 172, 172.4, 175.4; FT-IR ($CHCl_3$) 3008, 1736, 1600, 1449, 1224; HRMS calcd for

(25) Humphrey, J. M.; Bridges, R. J.; Hart, J. A.; Chamberlin, A. R. *J. Org. Chem.* **1994**, *59*, 2467.

(26) In order to synthesize both L- and D-α-amino dicarboxylates of six to eight carbon chain lengths with high enantiomeric purities we have developed an enantioselective hydrogenation approach reported in Pham, T.; Lubell, W. D. *J. Org. Chem.* **1994**, *59*, 3676.

$C_{31}H_{33}N_2O_4$ (MH^+) 497.2440, found 497.2423. Anal. Calcd for $C_{31}H_{32}N_2O_4$: C, 75; H, 6.5; N, 5.6. Found: C, 75.1; H, 6.5; N, 5.6.

Acknowledgment. This research was supported in part by the Natural Sciences and Engineering Research Council of Canada, and the Ministère de l'Éducation du Québec. The crystal structure analyses of compounds **4**–**6** were performed by Francine Bélanger-Gariépy at l'Université de Montréal X-ray facility. We are grateful for a gift of authentic ATPA from Dr. S. Treppendahl and Novo Nordisk. W.D.L. thanks Bio-Méga/Boehringer Ingelheim Recherche Inc. for a Young Investigator Award.

Supplementary Material Available: ^1H and ^{13}C NMR spectra of **1**–**6**, and ATPA (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO950111U