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TETRAHEDRON: *ASYMMETRY*

Highly enantioselective synthesis of a fluorescent amino acid

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Abstract

A high enantiomeric excess (>99.5%) synthesis of L-2-amino-3-(7-methoxy-4-coumaryl) propionic acid (L-Amp) is described. The two step synthesis route of this non-proteinogenic amino acid includes an oxazinone derivative as glycine enolate, which is alkylated with the fluorogenic group. © 2001 Published by Elsevier Science Ltd.

1. Introduction

Amp, a non-proteinogenic fluorescent amino acid is a fluorophore with a high quantum yield that can easily be built into peptides. Together with the 2,4-dinitrophenyl group it is often used as a fluorophore-quencher pair[1] or by itself, to label bioactive peptides.[2] In our studies on helical peptides we plan to label peptides with this fluorogenic amino acid. To keep the helical structure intact, however, a high enantiomeric excess of the label is needed. The previously reported method to synthesize Amp in enantiopure L-form afforded 94% enantiomeric excess (ee).[3,4] Our goal was to develop a synthetic approach that results in a higher enantiomeric excess of the L-form.

Chiral glycine equivalents serve as useful α-amino acid templates undergoing homologation via carbon–carbon bond formation at the α-position through nucleophilic carbanion alkylation.[5] Diphenyloxazinones are versatile templates[6,7] from which proteinogenic and non-proteinogenic amino acids can be synthesized in very high enantiomeric excess.[8]

2. Results and discussion

The synthesis of **3** (Scheme 1) started with the alkylation of benzyl (2*R*,3*S*)-(−)-6-oxo-2,3-diphenyl-4-morpholine-carboxylate, **1**: Sodium bis(trimethylsilyl)amide easily deprotonates **1** at

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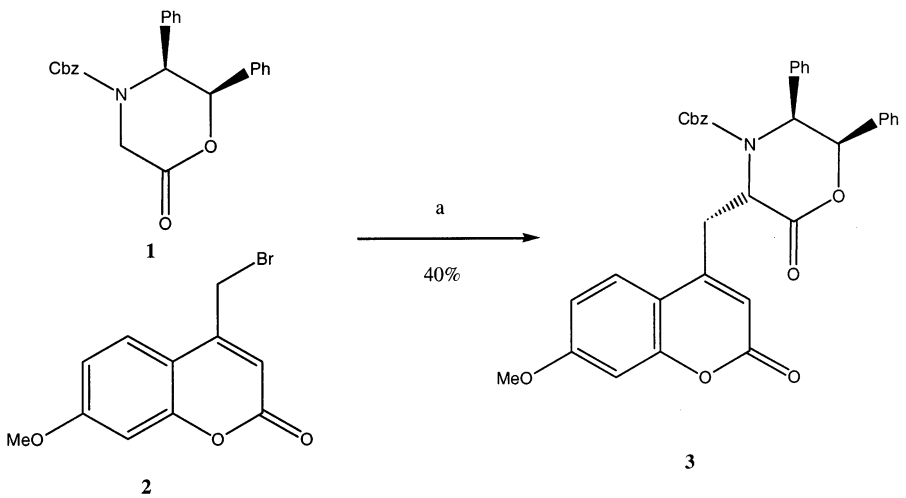
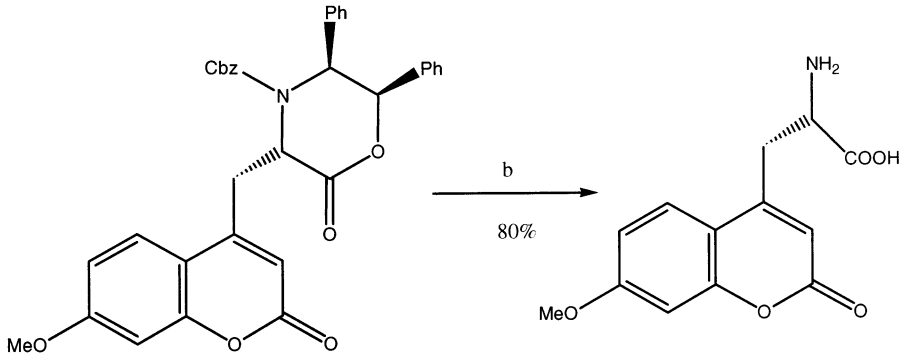
position 3. The enolate is then alkylated with 4-bromomethyl-7-methoxycoumarin, which yields **3**. Compound **2** is poorly soluble in THF at −78°C, and as the reaction mixture was cooled it precipitated. The enolate derived from **1**, however, was reactive enough to afford a satisfactory yield of **3**. As the reaction proceeded the precipitate disappeared, implying the formation of **3**, which was readily soluble in THF even at low temperature. The purification of the resulting compound, **3**, had some difficulties, since the originally followed procedure failed for this molecule. Finally, the method we developed gave the compound in pure form (Scheme 1). This intermediate was then hydrogenated to remove the benzyloxycarbonyl (Cbz) protecting group and set the amino acid **4** free from the oxazinone (Scheme 2). The conditions during this second reaction step were crucial. Not only should they remove the Cbz group and liberate the amino acid but they should leave the coumarin double bond intact. When THF/MeOH was used as a solvent during the hydrogenolysis with 0.7 equiv. of the catalyst, the double bond was found to be saturated and no fluorescence could be detected. Pure THF, however, with 0.5 equiv. catalyst gave the right product in good yield. The probable explanation for this result is that the Cbz group removal and the amino acid liberation occurs first then the amino acid precipitates in THF, which protects the double bond from saturation. For enantiomeric excess determination, racemic Amp and L-Amp, respectively, were condensed with *N*-(2,4-dinitro-5-fluorophenyl)-L-alaninamide.[9] The adducts were then checked by analytical HPLC. Within the detection limit of the instrument (0.5%) a single peak was detected that comigrated with one of the two adducts formed during the reaction with DL-Amp (Fig. 1).



Scheme 1. *Reagents*: (a) Sodium bis(trimethylsilyl)amide, THF, −78°C, 2 h; purified on PTLC (CH_2Cl_2/EtOAc 20:1 v/v%)

In summary, we have completed a synthesis of L-Amp in enantiopure form (>99.5% ee). Conformational studies on peptides labeled with this non-proteinogenic fluorescent amino acid and also synthesis of other fluorogenic amino acids in enantiopure form are in progress and will be reported in the near future.



3 4

Scheme 2. *Reagents*: (b) $PtCl_2$ (0.5 equiv.), H_2 (50 psi), THF, 24 h; filtered, concentrated, then triturated with Et_2O

3. Experimental

3.1. General

Compounds **1** and **2** were commercially available. ^1H NMR, and ^{13}C NMR: Bruker AC 300. IR: Perkin–Elmer 2000 FT-IR. HRMS: University of Illinois at Urbana-Champaign Mass Spectrometry Laboratory. Optical rotation: Perkin–Elmer 241 polarimeter. Analytical HPLC was carried out on a Waters Alliance 2690 instrument equipped with a Symmetry® C_{18} 3.5 μm 4.6×75 mm column.

3.2. (3S,5S,6R)-4-(Benzyloxycarbonyl)-5,6-diphenyl-3-[(7-methoxy-4-coumaryl)methyl]-2,3,5,6-tetrahydro-4H-1,4-oxazin-2-one **3**

Compounds **1** (216 mg, 0.56 mmol, 1.5 equiv.) and **2** (100 mg, 0.37 mmol, 1 equiv.) were dissolved in THF (20 ml) and cooled to the temperature of dry ice–acetone. To this stirred solution sodium bis(trimethylsilyl)amide (280 μl, 2 M solution in THF, 0.56 mmol, 1.5 equiv.) was added dropwise via syringe. After the addition of the base the mixture was stirred for two hours then the solution was allowed to warm up to room temperature and poured onto methylene chloride. The solution was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The yellowish solid was then purified on PTLC (eluted with CH_2Cl_2/EtOAc 20:1) allowing 86 mg of **3**, as a white powder (40%). ^1H NMR ($CDCl_3$, 393 K vs TMS): δ 3.29 (1H, dd, $J=9.64$ Hz, $J=13.37$ Hz), 3.79 (1H, dd, $J=4.82$ Hz, $J=13.17$ Hz), 3.88 (3H, s), 4.96 (1H, 1/2AB q, $J=12.34$ Hz), 5.05 (1H, 1/2AB q, $J=12.36$ Hz), 5.13 (2H, s), 5.41 (1H, dd, $J=4.86$ Hz, $J=9.52$ Hz), 5.83 (1H, d, $J=2.75$ Hz), 6.16 (1H, s), 6.5 (2H, d, $J=7.45$ Hz), 6.74 (2H, d, $J=7.1$ Hz), 6.86–6.95 (4H, m), 7.04–7.4 (8H, m), 8.05 (1H, d, $J=8.89$ Hz). ^{13}C NMR ($CDCl_3$): δ 36.65, 55.78, 56.93, 60.98, 68.02, 79.28, 79.46, 101.40, 112.36, 112.80, 114.12, 125.91, 126.41, 127.40, 127.46, 128.06, 128.22, 128.34, 128.88, 128.97, 133.50, 134.95, 135.18, 149.97, 154.64, 155.72, 160.61, 162.98, 166.65. IR (KBr) 1761.67, 1711.54, 1689.50; mp 215–216°C; $[\alpha]_D^{25}$ −25 (*c* 0.13, CH_2Cl_2); HRMS: calcd for $C_{35}H_{30}NO_7$: 576.2022 (HM^+), found: 576.2022 (HM^+).

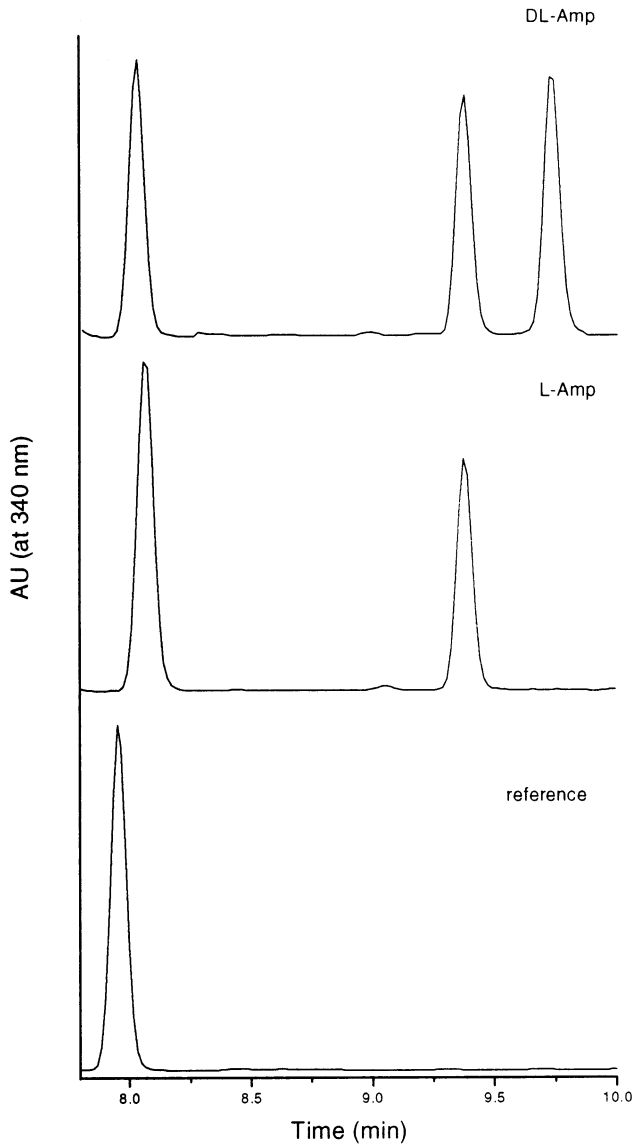


Figure 1. Analytical HPLC chromatograms of the products formed by reaction of *N*-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (bottom, used as reference), L-Amp (center), DL-Amp (top)

3.3. Hydrogenolysis

The oxazinone intermediate **3** was dissolved in THF and the solution was hydrogenated for 24 hours in the presence of 0.5 equiv. of $PtCl_2$ catalyst. Methanol was then added to dissolve the precipitated amino acid and the solution was filtered through Celite, then concentrated. The precipitate was triturated with Et_2O and the product was collected by centrifugation.

3.4. Formation of diastereomeric adducts

L-Amp and DL-Amp (0.5 mol), respectively, in 1 M $NaHCO_3$ (40 μl) and *N*-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (0.7 mol) in acetone (20 μl) were allowed to react for 1 hour at 40°C. After that 2 M HCl (20 μl) was added and the solutions were centrifuged. The supernatants were analyzed on analytical HPLC (solvents used: A, 0.1% trifluoroacetic acid in water; B, 0.08% trifluoroacetic acid in acetonitrile. A linear gradient of 5–95% B over 20 min at 1.25 ml/min. Elute monitored at 300–400 nm).

Acknowledgements

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