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An efficient synthesis of the intrinsic fluorescent peptide labels, (*S*)- and (*R*)-(6,7-dimethoxy-4-coumaryl)alanines via asymmetric hydrogenations

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Abstract—The title compounds **1a**,**b** are efficiently synthesized in high yields and with high enantioselectivity (>95% ee) by using a sequence in which the key step involves asymmetric hydrogenation of dehydroamino methyl ester **4** with Burk's DuPHOS-based Rh(I) catalysts.
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The utilization of fluorescent labeled peptides for biological assays often provides higher sensitivity than that observed when UV–vis detectable labels are employed. The selection of appropriate fluorophore moieties is a critical feature of the former method. Specifically, the fluorophore must absorb and emit at longer wavelength in order to reduce interference from other aromatic fluorophore moieties in amino acids.[1] Recently (*S*)-(6,7-dimethoxy-4-coumaryl)alanine (Dmca), has been prepared for use as an intrinsic fluorescent peptide label (Fig. 1).[2] This unusual amino acid has an absorption band at 345 nm and an emission maximum at ca. 440 nm.[2b] These unique spectroscopic properties enable selective determination of Dmca-labeled peptides even when tryptophan residues are present. Furthermore, the high quantum yield ($\phi = 0.52$) and large molar absorptivity ($\varepsilon = 10{,}900\ M^{-1}\,cm^{-1}$) of Dmca leads to selective detection of picomole quantities of labeled peptides. This level of detection sensitivity is comparable to that of radiolabeling.[2b]

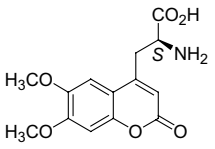

Figure 1. (*S*)-(6,7-Dimethoxy-4-coumaryl)alanine (Dmca).

The advantages of Dmca and its analogues have triggered a considerable recent interest in their syntheses and biological applications.[1–3] Two related methods, relying on the use of (2*R*)-bornane-10, 2-sultam-based and Williams' auxiliaries, have been developed for the highly diastereoselective preparation of L-Dmca.[1b,2a,3a] In addition, Dmca and its analogues have been incorporated into peptides of bovine neurotensin 8–13 fragment[3b] and fluorogenic peptide substrates[1b] for biological assays. In ongoing studies in our laboratory, we plan to use these fluorescent labels as part of an effort aimed at the development of peptide and peptidomimetic based inhibitors of X-linked inhibitor of apoptosis protein (XIAP). However, the lack of general and efficient methods to prepare both the D- and L-forms of the coumaryl-alanines inhibited this effort. From the perspective of cost, the reported auxiliary-based approaches are not ideal.[1b,2a,3a] In this letter, we describe an alternative, more cost-efficient method for the diastereospecific synthesis of both D- and L-forms of Dmca. The strategy is based on asymmetric hydrogenation of the dehydroamino ester **4** (Scheme 1), which independently provides the (*S*)- and (*R*)-enantiomers in a high enantioselective manner.

The synthesis of the (*S*)- and (*R*)-(6,7-dimethoxy-4-coumaryl)alanines started with commercially available

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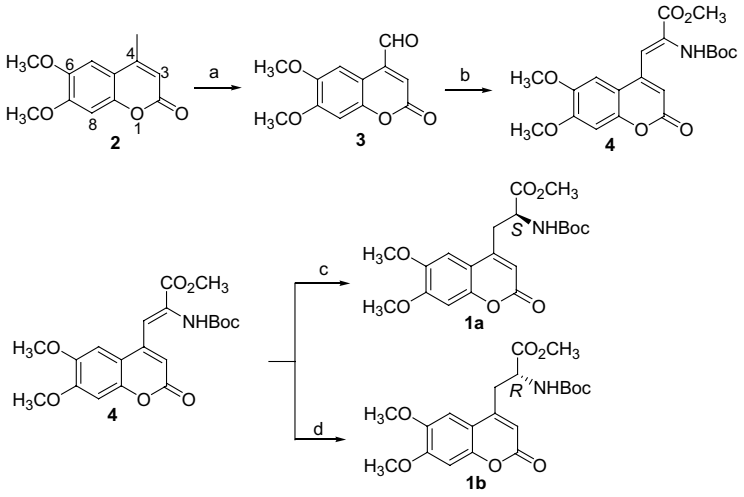

Scheme 1. Synthesis of (*S*)- and (*R*)-(6,7-dimethoxy-4-coumaryl)alanine derivatives (**1a**,**b**): Reagents and conditions: (a) SeO_2, xylene, reflux, 91%; (b) $(MeO)_2P(O)CH(NHBoc)CO_2Me$, DBU, CH_2Cl_2, 5 h, 77%; (c) H_2 (65 psi), [((*R*,*R*)-Et-Du-PHOS)-Rh]Tf, MeOH, 24 h, 96%; (d) H_2 (65 psi), [((*S*,*S*)-Et-Du-PHOS)-Rh]OTf, MeOH, 24 h, 99%.

6,7-dimethoxy-4-methylcoumarin (**2**) (Scheme 1). The methyl group in **2** was efficiently oxidized to an aldehyde in **3** by using selenium dioxide, following the literature procedure.[4,5] Horner–Emmons olefination of the resulting aldehyde with $(MeO)_2P(O)CH(NHBoc)COOMe$ in the presence of DBU gave the dehydroamino acid methyl ester **4** as a separable (silica gel) mixture of *Z*- (major, 95/5) and *E*-isomers.[6,7]

With **4** in hand, asymmetric hydrogenation reactions were explored. For this purpose, we chose 1,2-bis-((2*R*,5*R*)- and (2*S*,5*S*)-2,5-diethylphospholano)benzene-(cyclooctadiene)rhodium(I) trifluoromethane sulfonate ((*R*,*R*)/(*S*,*S*)-[Et-DuPHOS-Rh(COD)]OTf) as catalysts.[8] In early studies, we have demonstrated that reductions by using these catalysts proceed in high yields (>95%) and give single enantiomers (>97% ee) almost exclusively.[9] Moreover, these commercially available catalysts[10] display high turnover efficiency (catalyst to substrate ratios up to 1/2500).[8] In the current effort, we found that reduction employing the (*R*,*R*)-Et-DuPHOS containing catalyst afforded the amino acid derivative **1a** in an excellent level of enantioselectivity (95% ee) in an almost quantitative yield.[11,12] However, interestingly it was found that **1a** had an (*S*) absolute configuration, determined by converting it to known (*S*)-(6,7-dimethoxy-4-coumaryl)alanine [found: $[\alpha]_D$ +129.24 (*c* 0.5, DMF), lit.[3a] $[\alpha]_D$ +131.58 (*c* 0.0076, DMF)] (Fig. 1). This result is directly contrast to that observed, as reported in the literature, (*R*,*R*)-Et-DuPHOS ligand leading to (*R*)-product.[8a] The (*R*) amino acid **1b** was obtained in a high yield and high enantioselectivity (97% ee) when the (*S*,*S*)-Et-DuPHOS was used.[11,12] It is also interesting to note that the α,β-unsaturated lactone was not reduced under these hydrogenation conditions.

In summary, an efficient method for synthesis of highly enantiomerically enriched, coumarin-containing fluorescent amino acids has been developed. The key step in the route used for this purpose involves asymmetric hydrogenation reactions of the readily prepared dehydroaminoacid ester **4**. The incorporation of these amino acids into biologically active peptides and peptidomimetics is being investigated in our continuing work in this area.

Acknowledgements

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5. Compound **3**: ^1H NMR (500 MHz, $CDCl_3$): δ 10.05 (s, 1H), 8.09 (s, 1H), 6.89 (s, 1H), 6.76 (s, 1H), 3.97 (s, 3H) 3.97 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 192.5, 161.2, 153.7, 151.2, 147.2, 143.6, 123.4, 107.4, 106.5, 100.1, 56.6; HRMS (EI) exact mass calcd for $[M^+]$ ($C_{12}H_{10}O_5$) 234.0522, found 234.0539.
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7. Compound **4**: ^1H NMR (500 MHz, $CDCl_3$): δ 7.67 (s, 1H), 7.09 (s, 1H), 6.91 (s, 1H), 6.86 (s, 1H), 6.41 (s, 1H), 3.96 (s, 3H), 3.90 (s, 3H), 3.60 (s, 3H), 1.53 (s, 9H); ^{13}C NMR

(125 MHz, $CDCl_3$): 163.9, 161.6, 153.0, 152.6, 151.5, 149.6, 146.4, 130.0, 114.2, 111.9, 111.4, 106.5, 100.4, 82.0, 56.7, 56.6, 53.2, 28.5; HRMS (EI) exact mass calcd for $[M^+]$ ($C_{20}H_{23}NO_8$) 405.1418, found 405.1458.

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11. Enantiomeric excess was determined by Chiralcel OD-H column (0.46 cm × 25 cm), eluting with hexanes/isopropanol (90/10) at a flow rate of 1.5 mL/min. Essentially one peak was observed in each case. The retention time for (*S*)-**1a**: 20.2 min and (*R*)-**1b**: 24.8 min.
12. Compound **1a**: ^1H NMR (400 MHz, $CDCl_3$): δ 7.29 (s, 1H), 6.79 (s, 1H), 6.11 (s, 1H), 5.33 (br d, 1H, $J = 7.7$ Hz), 4.61 (dd, 1H, $J_1 = 6.8$ Hz, $J_2 = 14.0$ Hz), 3.94 (s, 3H), 3.90 (s, 3H), 3.67 (s, 3H), 3.06–3.19 (m, 2H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 161.2, 155.1, 152.4, 151.1, 149.8, 146.6, 113.3, 111.5, 105.4, 100.4, 80.6, 56.7, 56.4, 53.1, 52.8, 36.2, 28.3; HRMS (EI) calcd for $C_{20}H_{25}NO_8$ 407.1575; found 407.1540, $[\alpha]_D$ −18.28 (*c* 0.5, $CHCl_3$). Compound **1b**: ^1H NMR (400 MHz, $CDCl_3$): δ 7.29 (s, 1H), 6.79 (s, 1H), 6.06 (s, 1H), 5.34 (br d, 1H, $J = 7.8$ Hz), 4.61 (dd, 1H, $J_1 = 6.9$ Hz, $J_2 = 14.0$ Hz), 3.94 (s, 3H), 3.90 (s, 3H), 3.67 (s, 3H), 3.06–3.18 (m, 2H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 161.2, 155.2, 1153.0, 151.2, 149.8, 146.6, 113.3, 111.5, 105.4, 100.4, 80.6, 56.7, 56.4, 53.1, 52.8, 36.2, 28.3; HRMS (EI) calcd for $[M^+]$ ($C_{20}H_{25}NO_8$) 407.1575, found 407.1540; $[\alpha]_D$ +11.55 (*c* 1.0, $CHCl_3$).