Molecules **2007**, *12*, 1170-1182

molecules

ISSN 1420-3049

www.mdpi.org/molecules

Full Paper

Stereoselective Syntheses of Fluorescent Non-Natural Aromatic Amino Acids Based on Asymmetric Michael Additions

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Received: 10 May 2007; in revised form: 24 May 2007 / Accepted: 25 May 2007 / Published: 26 May 2007

Abstract: Four fluorescent non-natural aromatic amino acids have been synthesized based on a key stereoselective Michael addition reaction. *S*-1-Phenylethylamine was employed as both the source of amine and the stereoselectivity controller. The overall yields were moderate (30-50%). Fluorescent properties of some of the fluorophores were also investigated. It was found that compounds with a dimethylamino group bonded to the aromatic ring display intramolecular charge transfer fluorescence.

Keywords: Aladan, fluorescent non-natural amino acids, asymmetric Michael addition, stereoselective synthesis.

Introduction

Due to the development of the nonsense (stop codon) suppression technique, it has been possible to site-specifically incorporate any non-natural amino acid into almost any protein [1]. One of the significant features of this progress is that it offers a tool of almost limitless potential, promising chemical biologists systematic protein structure/function studies of great precision [2]. The development of fluorescent non-natural amino acids relates to this purpose. This new type of amino acid could play both the roles of structural moiety and intrinsic fluorescent probe when incorporated

into target bioactive molecules. Consequently, much attention has been focused on the synthesis of these novel amino acids. As an example, β-[6'-(*N*,*N*-dimethyl)amino-2'-naphthoyl]alanine [aladan (Ald)] was designed and first synthesized by Cohen's group and was further incorporated site selectively at buried and exposed sites in the B1 domain of protein G [3]. We are aware of several additional applications of aladan [4-5], including the one recently reported by our group [6].

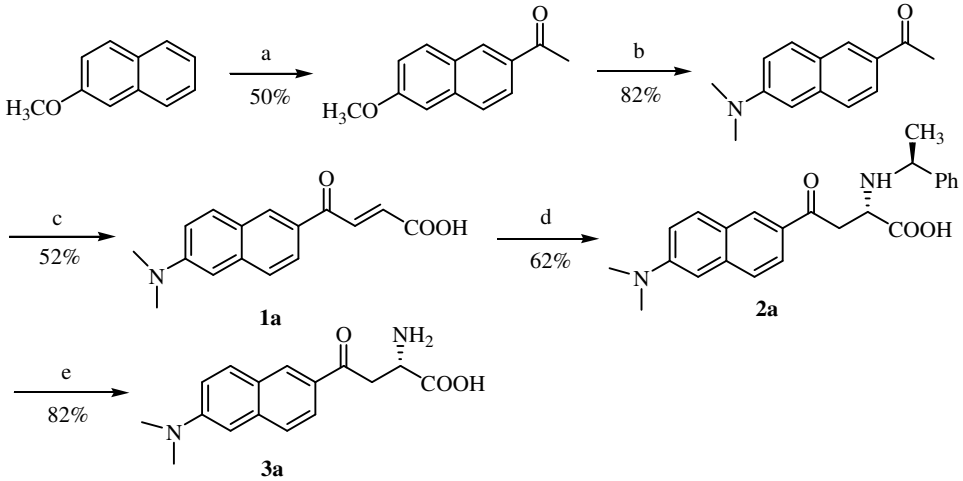
Cohen's group's first synthesis of aladan [3] was based on a core reaction sequence involving stereoselective condensation of 2-(α-iodoacetyl)-6-dimethylaminonaphthalene with *N*-(diphenylmethylene)glycine *tert*-butyl ester using *O*-(9)-allyl-*N*-(9-anthracenylmethyl) cinchonidinium bromide as both phase transfer catalyst and stereocontrol device. However, several drawbacks were noted when we repeated that synthesis. First, 2-(α-iodoacetyl)-6-dimethylaminonaphthalene was sensitive to light, moisture and temperature, which is certainly inconvenient, especially in the workup step. The second was that in order to guarantee the stereoselectivity, more than 20 mol% of the phase transfer catalyst had to be used and a long reaction time (>24 hrs) at a very low temperature (-78 °C) avoiding light was required. Moreover, usage of 1,2-dithioethanol as auxiliary reagent to deprotect the diphenylmethylene group can generate environmental problems. Finally, the overall yield was not high (only 30%). Consequently, we began to conceive a new scheme to prepare the title compounds (Scheme 1). Moreover, this scheme has also been applied to the synthesis of several other non-natural amino acids.

Results and Discussion

Synthesis of non-natural fluorescent amino acids

As displayed in Scheme 1, steps (a) and (c) followed the same procedure as described in our previous work [6]. In step (c), *E-6'*-(*N*,*N*-dimethylamino)-*2'*-naphthoylacryl acid was prepared via an aldol reaction using a solution of tetramethylamine hydroxide in methanol as base and then immediately dehydrated. The yield in this step was moderate (52%).

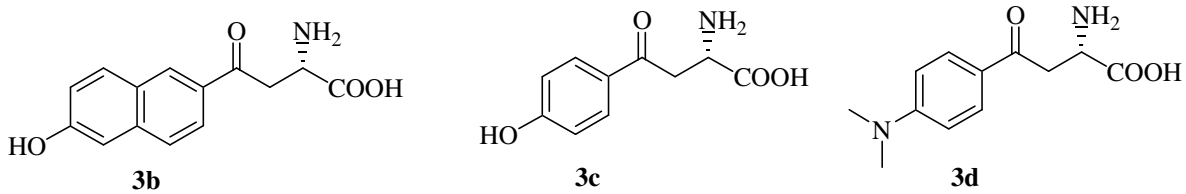
Scheme 1. New synthesis of aladan.



(a) acetyl chloride, nitrobenzene, 0°C → r. t., 24 h; (b) 5 eq $Li/HN(CH_3)_2$, dry benzene/HMPA, r.t., 24 h; (c) OHCCOOH, 20 wt% $(CH_3)_4N^+OH^-$ in methanol, 0 °C → r. t.; followed by refluxing in *p*-TosOH/benzene; (d) *S*-1-phenylethylamine, ethanol, 50-60 °C, 16 h; (e) 6N HBr, reflux, 12 h.

Step (d) is the key reaction of our new scheme. *S*-1-Phenylethylamine is used both as the amine source of aladan and the stereocontroller. The reaction was carried out at a temperature of 50 to 60 °C for 16 hours. The title compound, (α*S*,1*S*)-α-[*N*-(1-methyl)benzyl]-β-[(6'-dimethylamino)-2'-naphthoyl]alanine (**2a**), began to precipitate from hot ethanol one hour later; as pointed out by Yamada *et al*. [7], this dynamic preference was an important factor to resolve the (α*S*,1*S*) isomer and made it the major product. The workup, in which only filtration and washing with cold ethanol are used, was quite simple. Analytical results from RP-HPLC and HPLC on a chiral column (Chirex 3005, Phenomenex®) shown that purity and *de* value of the title compound was quite satisfactory (Table 2). The yield was 62.0%. Removal of *N*-(1-methyl)benzyl was carried out in satisfactory yield by refluxing the α-amino protected compound in 6N HBr. The absolute configuration of compound **3a** was confirmed as *S* by comparing the results of RP-HPLC and chiral HPLC analysis with those of the standard sample prepared by Cohen's method [3]. Scheme 1 has also been successfully applied to synthesize several other non-natural amino acids (**3b ~ 3d**, Figure 1). The yields were moderate.

Figure 1.



3b **3c** **3d**

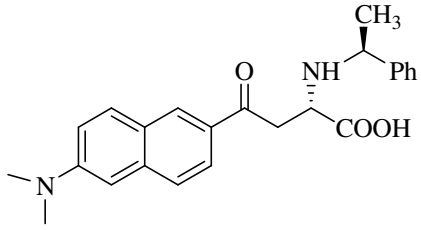
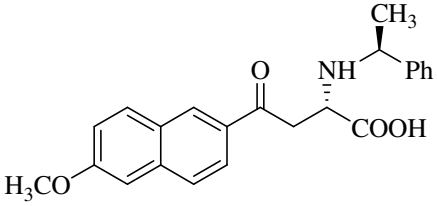
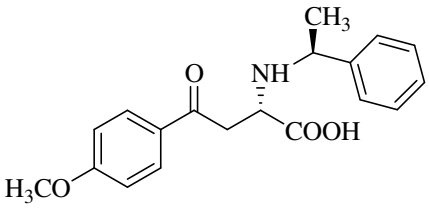
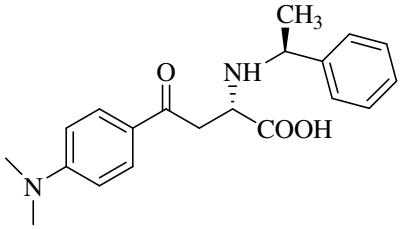
Efforts have also been made to prepare *E*-acryl acids **1a~1d** by direct Friedel-Crafts reactions using maleic anhydride as the starting material under anhydrous $AlCl_3$ catalysis. Unfortunately, only compound **1c** could be successfully prepared by this method using 1,1,2,2-tetrachloroethane as solvent. The yield was 60.5%.

Table 1 displays the results for synthesizing compounds **2a~2d** via asymmetric Michael reaction. It was shown that the yields for compounds **2b** and **2c** were higher than those for compounds **2a** and **2d**. The reasons might be that with a dimethylamino group replacing the methoxyl group in the corresponding site of *E*-acryl acid substrate, the nucleophilicity for Michael addition reaction was lowered down due to the stronger combination of dimethylamino group to aluminum chloride.

The *S*-configuration of compound **2c** was explained by Yamada *et al*. [7]. Therefore, the *S* configurations of **2b** and **2d** were confirmed by a reasoned comparison of **2b** with **2a**, and **2d** with **2c**, based on combined results of reaction mechanism, RP-HPLC and chiral HPLC analysis.

The four free non-natural fluorescent amino acids were easily turned into the corresponding *N*-Fmoc protected derivatives by the interaction of 9-fluorenylmethyl chloroformate at room temperature. The products were designated as **4a** to **4d**, respectively, according to the above order. The yields were high, which lay between 82 to 93%. The N-acetyl amides are prepared followed a solid phase synthesis assay. An amide type resins, TentaGel S RAM resins were used. The resulting derivatives were therefore named **5a** to **5d** (Table 2).

Table 1. Data of yield, purity, and *de* values for (α*S*, 1*S*)-α-[*N*-(1-methyl)benzyl]alanine derivatives **2a ~ 2d** prepared via asymmetric Michael reactions *.

Entry	Structure	Yield (%)	Purity (%)	*de* value (%)
2a		62	98	95
2b		76	99	96
2c		86	99	97
2d		63	97	95

*calculation of yield was based on the amounts of precipitate; calculation of the purity and *de* values were based on results from both RP-HPLC and chiral HPLC, respectively.

Fluorescence studies

Investigation of the steady-state fluorescence for the synthetic compounds had been carried out. As shown in Table 2 and Figure 2, when the methoxyl group in 6-methoxyl-2-acetylnaphthalene was replaced with a dimethylamino group, the maximum UV absorption and maximum fluorescent emission wavelength red-shifted 50 and 78 nm, respectively. This means that dimethylamino group is a better electron-donor group than a methoxyl group; it can affect the energy distribution of the molecular orbital to a larger degree. On the other hand, the quantum yield of acedan (6-dimethylamino-2-acetylnaphthalene) was almost three times lower that that of 6-methoxyl-2-acetylnaphthalene. This phenomenon was ascribed to the fact that the dimethylamino moiety can increase the rate of nonradiative decay (k_{nr}).

Quite interestingly, it was found that all three compounds, *p*-dimethylaminoacetopheneone, Ac-Ald-NH_2 and Ac-Alb-NH_2 (β-[4-(*N*, *N*-dimethyl)aminobenzoyl]alanine, **Alb**), displayed intramolecular charge transfer dual fluorescence in Tris-HCl buffer solution. Compared to a similar fluorophore,

p-dimethylaminobenzoylamide, which displayed strong charge transfer (CT) fluorescence at 480 nm and a weak locally excited (LE) fluorescence at 350 nm (an experiment in which acetonitrile was used as solvent) [8-10]; whilst in the case of *p*-dimethylaminoacetopheneone, it displayed a strong CT fluorescence at 414 nm and a weak LE fluorescence at 554 nm.

When acedan was converted into Ac-Ald-NH_2, the UV maximum absorption wavelength was almost the same (from 361 nm to 364 nm), but the fluorescent maximum emission wavelength red-shifted 15 nm. In the meantime, the quantum yield decreased by about two-fold and a new LE fluorescence at 440 nm appeared. This revealed that the introduction of a glycyl moiety into acedan increased the possibility of nonradiative decay. A same phenomenon was found when a glycyl moiety was introduced into *p*-dimethylaminoacetophenone.

Table 2. Fluorescence spectroscopic parameters for some compounds in Tris-HCl (20 mmol/L, pH 6.6, 20 °C) (λ_{exc} = 350 nm).

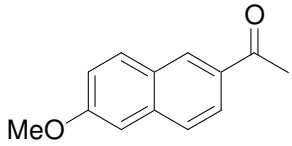
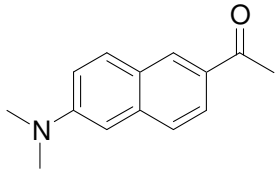
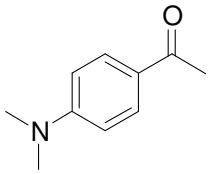
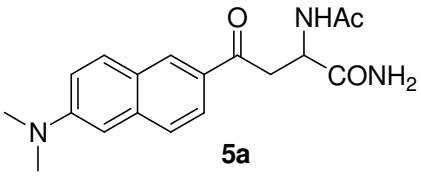
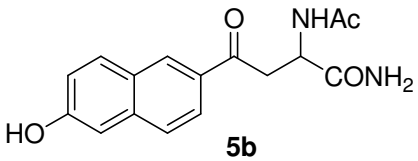
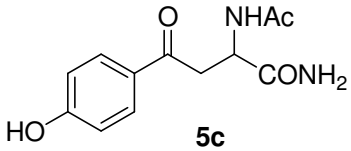
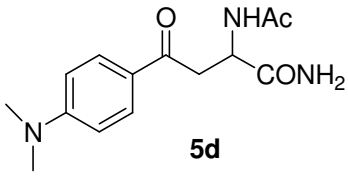
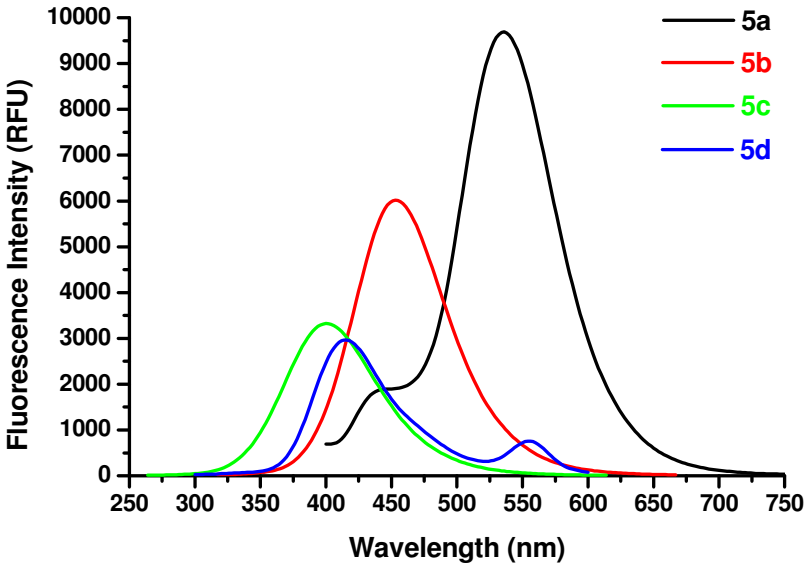
Compd. structure	Abs λ_{max} (nm)	E_m λ_{max} (nm)	ϕ
	311	444	0.392
	361	522	0.143
	301	414(s), 554(w)	0.112
 **5a**	364	440(w), 539(s)	0.069
 **5b**	316	456	0.106
 **5c**	281	403	0.076
 **5d**	305	432(s), 572(w)	0.053

Figure 2. Fluorescence spectra of four *N*-acetyl amides.



Determinations were carried out in Tris-HCl (20 mmol/L, pH 6.6, 20 °C) (λ_{exc} = 350 nm)

Conclusions

In summary, an efficient and simple alternative for the preparation of aladan and three other non-natural fluorescent amino acids via asymmetric Michael addition using *S*-1-phenylethylamine was reported. Prominent among the advantages of this new method are operational simplicity, inexpensive reagents, shorter reaction time, higher overall yield, high stereoselectivity and easily workup. It was disclosed that acedan, *p*-dimethylaminoacetophenone, Ac-Ald-NH_2, Ac-Alb-NH_2 display intra-molecular charge transfer fluorescence.

Experimental

General

All chemicals were purchased from Aldrich Chemical Co. Nitrobenzene was freshly distilled from 4Å molecular sieve; Benzene, HMPA, and 1,1,2,-tetrachloroethane were dried over 4Å molecular sieve; THF was freshly distilled from sodium granules. Other reagents were used as supplied. Melting points were determined on an Electrothermal melting point apparatus. Both ^{1}H-NMR and ^{13}C-NMR were acquired on Varian VXG-400 S equipment. Mass spectra was recorded on either VG-MM-1212 or MS 902/ZAB instruments. Optical rotations were measured on a Perkin Elmer Polarimeter 343 (PerkinElmer INC., USA). HPLC was carried on a Varian LC Star system (Varian Associates Inc., USA); The column used for reversed-phase analysis was purchase from Vydac (Hesperia, CA 92345, USA), size: 0.46×25 cm packed with 5 μm C_{18} modified silica gel; the chiral column was available from Phenomenex® (Torrance, CA, USA), model: Chirex (R)-NGLY and DNB; size: 0.46×25 cm. Method used for RP-HPLC analysis: isocratic using pH 2.5 aqueous phosphate buffer/acetonitrile (8/2,

V/V); flow rate: 0.5 mL/min; wavelength: 280 nm; Method used for chiral HPLC: isocratic using 0.03 mol/L NH_4Ac in methanol; flow rate: 0.5 ml/min; wavelength: 280 nm.

Fluorescence determinations

The determinations were carried out on a Gemini EM instrument (Molecular Devices Corporation, Sunnyvale, USA). The excitation wavelength was 350 nm, the cutoff was 420 nm, and the step was 2 nm. The spectral resolution was 2 nm for excitation and for emission. Solutions of sample compounds and reference amino acid in Tris/HCl buffer (pH 6.6) at a concentration of 2×10^{-5} mol/L were used. Fluorescence quantum yields (ϕ) were determined with *N*-acetyl-*L*-tryptophanamide (NATA) (ϕ_{NATA} = 0.14) as reference. The quantum yield was calculated based on the following equation:

$$\phi_S = \phi_R \frac{E_S A_R}{E_R A_S}\left(\frac{n_S}{n_R}\right)^2$$

where the subscripts *S* and *R* refer to the sample and reference compound (NATA), respectively. *E* is the integrated area under the corrected emission spectrum. *A* is the absorbance of the solution at the excitation wavelength ($A < 0.05$) and $(n_S/n_R)^2$ is the correction for the refractive index.

Synthesis of 6-methoxy-2-acetylnaphthalene

After following a procedure described in literature [6] the title compound was separated and purified by flash liquid chromatography (ethyl acetate/*n*-hexane, 1:4, *v/v*). The fraction corresponding to a R_f value of 0.42 was collected and further purified by crystallization (*n*-hexane) to give 12.6 g (50%) of the title compound as colorless plates. Mp 106-108°C (lit. [11] 104-105°C); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.39 (d, 1H, *J*=1.5 Hz), 8.0 (dd, 1H, *J*=8.6, 1.8 Hz), 7.85 (d, 1H, *J*=8.8 Hz), 7.76 (d, 1H, *J*=8.6 Hz), 7.2 (dd, 1H, *J*=8.9, 2.5 Hz), 7.15 (d, 1H, *J*=2.4 Hz), 3.95 (s, 3H), 2.7 (s, 6H); ^{13}C-NMR (100.6 MHz, CDCl3) δ 198.1, 160.0, 137.5, 132.8, 131.3, 130.3, 128.0, 127.3, 124.8, 119.9, 105.9, 55.6, 26.8.

Synthesis of 6-N,N-dimethyamino-2-acetylnaphthalene (acedan)

A literature procedure was followed [6]. The title compound was separated and purified by flash liquid chromatography (ethyl acetate/*n*-hexane, 3:7, *v/v*). The fraction with a R_f value of 0.49 was then further purified by crystallization (*n*-hexane/chloroform) to give 4.4 g (82%) of the title compound as needle-like crystals; mp 154-156°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.3 (s, 1H), 7.92 (dd, 1H, *J*=8.7, 1.8 Hz), 7.79 (d, 1H, *J*=9.2 Hz), 7.63 (d, 1H, *J*=8.6 Hz), 7.17 (d, 1H, *J*=6.2 Hz), 6.9 (s, 1H), 3.1 (s, 6H), 2.66 (s, 3H); ^{13}C-NMR (106 MHz, $CDCl_3$) δ 197.9, 150.0, 137.8, 131.2, 131.0, 130.6, 126.5, 125.2, 124.8, 116.5,105.8, 40.8, 26.7.

Synthesis of 4'-methoxyacetophenone

Anhydrous powdered $AlCl_3$ (20 g, 0.15 mol) and acetyl chloride (10.7 mL, 0.15 mol) were suspended in dried 1,1,2,2-tetrachloroethane (100 mL). The suspension was cooled down to 0 °C in an

ice bath, then anisole (10.8 mL, 0.1 mol) was added via syringe. The mixture was stirred at 0 °C for another 4 hours and then warmed up to room temperature and stirred overnight. The reaction was terminated by pouring the mixture into a solution of 4 M hydrochloric acid (100 mL) mixed with cracked ice. The suspension was then extracted with ethyl acetate (3×50 mL). All the extracts were combined and washed with water (3×20 mL) and brine (2×20 mL), respectively. The organic layer was then dried over anhydrous $MgSO_4$. After filtration and removal of ethyl acetate, the residue was purified by flash liquid chromatography (ethyl acetate/*n*-hexane, 3:7, *v/v*). The fraction with an R_f value of 0.51 was collected to give 13.6 g (91%) of the target compound as oil, which gradually solidified. Mp 37-39 °C (lit. [12] 36-38 °C); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, 2H, *J* = 1.5, 9.0 Hz), 6.90 (dd, 2H, *J* = 1.5, 9.0 Hz), 3.84 (s, 3H), 2.53 (s, 3H); ^{13}C-NMR (106 MHz, $CDCl_3$) δ 197.0, 163.7, 130.8, 130.5, 113.9, 44.8, 25.6.

Synthesis of 4'-(N, N-dimethyl)aminoacetophenone

The same procedure described for the synthesis of acedan was used employing 4'-methoxy-acetophenone (3.42 g, 22.8 mmol). The crude residue was separated and purified by flash liquid chromatography (ethyl acetate/*n*-hexane, 3:7, *v/v*). The fraction with R_f 0.45 was collected to give 1.213 g (33%) of the title compound as a light yellow solid. Mp 103-105 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.89 (d, 2H, *J* = 8.6 Hz), 6.68 (d, 2H, *J* = 8.8 Hz), 3.06 (s, 6H), 2.51 (s, 3H); ^{13}C-NMR (106 MHz, $CDCl_3$) δ 196.6, 153.4, 130.7, 111.2, 40.5, 26.2.

Typical experimental procedure for the synthesis of E-acryl acids derivatives via an aldol reaction

Aryl 2-acetyl acetone (10 mmol) and glyoxylic acid monohydrate (0.92 g, 10 mmol) were stirred in dried methanol and 20 wt% tetramethylammonium hydroxide in methanol (5.8 mL, 11 mmol) was added via syringe. The temperature gradually rose to room temperature and the mixture was stirred for 24 h. The reaction mixture was then poured into 2 M aqueous H_3PO_4 solution (20 mL) mixed with cracked ice. The mixture was concentrated to a volume of about 20 mL under reduced pressure at 39 °C. The residues were then extracted with chloroform (3×30 mL). All the extracts were combined and washed with water (3×10 mL) and brine (2×10 mL). The organic layer was then dried over anhydrous $MgSO_4$. Removal of chloroform was followed by reduced pressure evaporation at room temperature. To the residue *p*-toluenesulfonic acid (0.2 g, 1 mmol) and benzene (50 mL) were added, the flask was equipped with a Dean-Stark trap and the solution was refluxed for 4 h, then the benzene was removed by rotatory evaporation under reduced pressure at r.t. The residues was dissolved in chloroform (50 mL) and washed with water (3×10 mL) and brine (2×10 mL). The chloroform layer was then dried over anhydrous $MgSO_4$. After removal of chloroform, the residues were purified by liquid chromatography (EtOAc/*n*-hexane/HOAc, 60/38/2, *v/v/v*).

6'-(N,N-Dimethylamino)-2'-naphthoylacryl acid (**1a**). R_f = 0.63; deep red solid; yield: 52%; Mp. 144-146 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.60 (d, 1H, *J* = 1.5 Hz), 8.08 (d, 1H, *J* = 15.6 Hz), 7.97 (d, 1H, *J* = 9.2 Hz), 7.88 (m, 1H), 7.71 (m, 1H), 7.28 (m, 1H), 6.95 (m, 1H), 6.69 (d, 1H, *J* = 15.6 Hz), 3.06 (s, 6H); ^{13}C-NMR (106 MHz, DMSO-d_6) δ 188.2, 167.3, 151.3, 138.4, 137.1, 132.5, 131.9, 130.0,

127.2, 126.6, 125.0, 124.8, 117.1, 106.9, 40.7; FAB-MS *m/z*: calcd. for $C_{16}H_{14}NO_3$ for $[M-H]^+$ 268.11; found 268.1; daughter peak: 224, 209, 196, 181.

6'-Methoxy-2'-naphthoylacryl acid (**1b**). R_f = 0.64; yellow solid; yield: 70%; Mp. 135-136 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.75 (d, 1H, J = 1.5 Hz), 7.88-8.11 (m, 4H), 7.38-7.42 (m, 1H), 7.22-7.28 (m, 1H), 6.72 (d, 1H, *J* = 15.6 Hz), 3.9 (s, 3H); ^{13}C-NMR (106 MHz, DMSO-d_6) δ 198.3, 189.2, 176.4, 167.1, 160.6, 160.1, 136.9, 133.2, 132.4, 128.3, 128.1, 125.0, 120.4, 106.9, 56.2; FAB-MS *m/z*: calcd. for $C_{15}H_{13}O_4$ ($[M+H]^+$) 257.07, found 257.1; daughter peak: 211.07, 183.07, 159.07, 144.05.

p-Methoxybenzoylacrylic acid (**1c**). R_f = 0.5; white solid; yield: 66%; Mp. 126-128 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.03 (m, 3H), 7.01 (m, 2H), 6.86 (d, 1H, *J* = 15.6 Hz), 3.90 (s, 3H); ^{13}C-NMR (106 MHz, $CDCl_3$) δ 187.6, 171.0, 164.6, 138.8, 131.7, 131.0, 129.7, 114.5, 55.9; FAB-MS *m/z*: calcd. for $C_{11}H_{10}O_4$ ($[M+H]^+$) 207.19, found 207.17.

4'-(N, N-Dimethyl)benzoylacryl acid (**1d**). R_f = 0.43; golden solid; yield: 63%; Mp. 146-148 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.89 (m, 2H), 7.68 (m, 1H), 6.75 (m, 2H), 6.60 (d, 1H, *J* = 15.4 Hz), 3.03 (s, 6H); ^{13}C-NMR (106 MHz, DMSO-d_6) δ 186.1, 167.4, 154.5, 137.3, 131.7, 131.6, 130.8, 124.4,111.7, 40.5; FAB-MS *m/z*: calcd. for $C_{12}H_{13}NO_3$ ($[M+H]^+$) 219.24, found 219.21; daughter peak: 174.1, 146.1.

Typical experimental procedure for the synthesis of compounds **2a~2d** *by asymmetric Michael addition.*

To a vigorously stirred solution of *trans*-acrylic acid **1a~1d** (2.0 mmol) in ethanol (60 mL) at 50 to 60 °C, *S*-1-phenylethylamine (0.28 mL, 2.2 mmol) was added dropwise via syringe. After the addition was complete, the mixture was stirred at the same temperature for 24 h. Precipitation was first observed ca. 1 h into the reaction and increased as time went on. The mixture was then cooled down to room temperature, and the precipitates were collected by filtration and washed with cold ethanol. The precipitates were quite pure, judged from both RP-HPLC and chiral HPLC analytical results. Further purification by re-crystallization from acetonitrile/aqueous phosphate buffer (pH 2.5) was also performed.

(αS,1S)-α-[N-(1-Methyl)benzyl]-β-[(6'-dimethylamino)-2'-naphthoyl]alanine (**2a**). ^{1}H-NMR (400 MHz, DMSO-d_6/TFA) δ 8.43 (d, 1H, *J*=1.5 Hz,), 7.90 (d, J = 9.3 Hz, 1H), 7.80 (dd, 1H, *J*=8.8, 1.8 Hz) 7.68 (d, 1H, *J*=8.8 Hz), 7.40-7.54 (m, 5H), 7.28 (dd, 1H, *J*=9.2, 2.5 Hz), 6.97 (d, 1H, *J*=2.4 Hz), 4.58 (m, 1H), 3.93 (m, 1H), 3.72 (m, 2H), 3.06 (s, 6H), 1.61 (d, 3H, *J*=6.8 Hz); ^{13}C-NMR (100.6 MHz, DMSO-d_6/TFA) δ 194.6, 170.5, 150.8, 138.2, 137.0, 131.5, 131.3, 129.9, 129.7, 128.6, 126.9, 125.2, 124.4, 117.3, 114.4, 105.8, 58.7, 53.5, 40.5, 20.6; FAB-MS *m/z*: calcd. for $C_{24}H_{27}N_2O_3$ ($[M+H]^+$) 391.19, found 391.21; daughter peaks: 287, 214, 198, 172, 105; $[\alpha]_D^{20}$ = +94.7 ° (c = 0.21, methanol/0.1 mol/L H_2SO_4, 3/1, *v/v*).

(αS,1S)-α-[N-(1-Methyl)benzyl]-β-[(6'-methoxy)-2'-naphthoyl]alanine (**2b**). ^{1}H-NMR (400 MHz, DMSO-d_6/TFA) δ 8.57 (s, 1H), 8.01 (d, 1H, *J*=9.2 Hz), 7.90 (m, 2H), 7.52 (d, 1H, *J*=8.0 Hz), 7.42 (m, 5H), 7.24 (m, 1H), 4.58 (m, 1H), 3.89 (s, 1H), 3.88 (s, 3H), 3.68 (m, 2H), 1.62 (d, 3H, *J*=6.8 Hz); ^{13}C-NMR (100.6 MHz, DMSO-d_6/TFA) δ 195.2, 170.4, 160.4.9, 137.9, 136.9, 131.9, 131.1, 129.9, 129.7, 128.6, 128.0, 127.8, 124.6, 120.4, 120.1, 117.2, 114.3, 111.4, 106.6, 58.7, 55.9, 53.4, 20.5; FAB-MS *m/z*: calcd. for $C_{23}H_{24}NO_4$ ([M+H]$^+$) 378.16, found 378.2; daughter peaks: 274, 201, 185, 159, 105; $[\alpha]_D^{20}$ = +85.5 ° (c = 0.47, methanol/0.1 mol/L H_2SO_4, 3/1, *v/v*).

(αS,1S)-α-[N-(1-Methyl)benzyl]-β-[(4'-methoxy)benzoyl]alanine (**2c**). ^{1}H-NMR (400 MHz, DMSO-d_6/TFA) δ 7.92 (dd, 2H, *J*=1.6, 8.8 Hz), 7.40-7.51 (m, 5H), 7.03 (dd, 2H, *J*=1.6, 8.8 Hz), 4.59 (m, 1H), 3.89 (m, 1H), 3.82 (s, 3H), 3.61-3.46 (m, 2H), 1.60 (d, 3H, J = 6.6 Hz); ^{13}C-NMR (100.6 MHz, DMSO-d_6/TFA) δ 194.0, 170.4, 164.4, 136.9, 131.2, 129.9, 129.7, 128.8, 128.6, 120.1, 117.2, 114.6, 114.3, 111.4, 58.7, 56.1, 53.4, 20.5; FAB-MS *m/z*: calcd. for $C_{19}H_{22}NO_4$ ([M+H]$^+$) 328.15; found 328.2; $[\alpha]_D^{20}$ = +63.9 ° (c = 0.49, methanol/0.1 mol/L H_2SO_4, 3/1, *v/v*)

(αS,1S)-α-[N-(1-Methyl)benzyl]-β-[(4'-dimethylamino)benzoyl]alanine (**2d**). ^{1}H-NMR (400 MHz, DMSO-d_6/TFA) δ 7.75 (d, 2H, *J*=9.0 Hz), 7.38-7.50 (m, 5H), 6.68 (d, 2H, *J*=9.0 Hz), 4.59 (m, 1H), 3.82 (s, 1H), 3.41 (m, 2H), 2.98 (s, 6H), 1.59 (d, 3H, *J*=6.9 Hz); ^{13}C-NMR (100.6 MHz, DMSO-d_6/TFA) δ 192.7, 170.5, 154.3, 136.9, 130.8, 129.9, 129.7, 128.5, 123.2, 120.0, 117.1, 114.3, 111.4, 58.7, 53.6, 40.4, 20.5; *m/z* (FAB-MS): calcd. for $C_{20}H_{25}N_2O_3$ ([M+H]$^+$) 341.18; found 341.15; daughter peak: 237, 220, 164, 148, 122, 105, 88, 74; $[\alpha]_D^{20}$ = +113.2° (c=0.28, methanol/0.1 mol/L H_2SO_4, 3/1, V/V)

Typical deprotection procedure

To α-[*N*-(1-S-methyl)benzyl]alanine derivatives **2a~2d** (2 mmol) in a 100 mL flask, 6 N aqueous HBr solution (30 mL) was added. The flask was wrapped with aluminum foil. Under stirring, the mixture was kept refluxing for 12 h. After cooling down, the mixture was concentrated to sticky oil under reduced pressure at 45 °C and cold water (50 mL) was added. The precipitates were collected by filtration and washed carefully with cold water, acetone and ether and then further purified by crystallization using hot water.

(αS)-β-[6'-(N,N-Dimethyl)amino-2'-naphthoyl]alanine (**3a**). Yield: 0.61 g (82%); ^{1}H-NMR (400 MHz, D_2O+DCl) δ 8.68 (s, 2H), 8.19 (d, 1H, *J*=8.1 Hz), 7.96 (s, 2H), 7.78 (d, 1H, *J*=6.8 Hz), 4.35 (m, 1H), 3.84 (m, 2H), 3.16 (s, 6H); FAB-MS *m/z*: calcd. for $C_{16}H_{19}N_2O_3$ ([M+H]$^+$) 287.33; found 287.1. $[\alpha]_D^{20}$ = -42.3° (c=0.23, 1 mol/L HCl).

(αS)-β-(6'-Hydroxy-2'-naphthoyl)alanine (**3b**). Yield: 0.59 g (87%); ^{1}H-NMR (400 MHz, D_2O+DCl) δ 8.68 (s, 1H), 8.13 (d, 1H, *J*= 9.2 Hz), 8.02 (m, 2H), 7.63 (d, 1H, *J*=8.0 Hz,), 7.35 (m, 1H), 4.35 (m, 1H), 3.73 (m, 2H); FAB-MS *m/z*: calcd. for $C_{14}H_{14}NO_4$ ([M+H]$^+$) 260.08; found 260.10. $[\alpha]_D^{20}$ = -35.3° (c=0.45, 1 mol/L HCl).

(αS)-β-[(4'-Hydroxy)benzoyl]alanine (**3c**). Yield: 0.52 g (91%); ^{1}H-NMR (400 MHz, D_2O+DCl) δ 8.02 (dd, 2H, *J*=1.6, 8.8 Hz), 7.13 (dd, 2H, *J*=1.6, 8.8 Hz), 4.36 (m, 1H), 3.71 (m, 2H); FAB-MS *m/z*: calcd. for $C_{10}H_{11}NO_4$ ([M+H]$^+$) 210.71; found 210.65. $[\alpha]_D^{20}$ = -17.6° (c=0.5, 1 mol/L HCl).

(αS)-β-[(4'-Dimethylamino)benzoyl]alanine (**3d**). Yield: 0.53 g (83%); ^{1}H-NMR (400 MHz, D_2O+DCl) δ 7.86 (d, 2H, *J*=9.0 Hz), 6.89 (d, 2H, *J*=9.0 Hz), 4.36 (m, 1H), 3.52 (m, 2H), 3.11 (s, 6H); FAB-MS *m/z*: calcd. for $C_{10}H_{12}NO_4$ ([M+H]$^+$) 237.12; found 237.05. $[\alpha]_D^{20}$ = -25.3° (c=0.52, 1 mol/L HCl).

Typical experimental Fmoc-proctection procedure

To the above free amino acid HBr salt (2.0 mmol) dissolved in DMF (20 mL), aqueous Na_2CO_3 solution (10 mL, 0.53 g, 5.0 mmol) was added. The mixture was cooled down to 0 °C with an ice-water bath. 9-Fluorenylmethyl chloroformate (0.62 g, 2.4 mmol) was added in small portions under stirring in the ice-water bath for 4 hr. The solution was then warmed up to room temperature and stirred for another 8 hr. After the end of reaction, the mixture was poured into solution of crushed ice and water (100 mL) and concentrated hydrochloric acid was added slowly until the pH value was around 1~2. The mixture was extracted with chloroform (150 mL). The organics was washed with water (2×30 mL) and brine, respectively, and dried over anhydrous $MgSO_4$. After removal of solvent by rotary evaporation, the residues were separated and purified by flash liquid chromatography with ethyl acetate in chloroform (3:7, v/v).

α-N-Fmoc-(αS)-β-[6'-(N,N-Dimethyl)amino-2'-naphthoyl]alanine (**4a**). R_f = 0.62. Yield: 0.84 g (83%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.84 (br, 1H), 7.68 (m, 3H), 7.55 (m, 3H), 7.52-7.12 (m, 5H), 6.84 (s, 1H), 6.10 (s, 1H), 4.84 (br, 1H), 4.35-4.16 (m, 3H), 3.88 (m, 1H), 3.62 (m, 1H), 3.09 (s, 6H); ^{13}C-NMR (106 MHz, $CDCl_3$) δ 163.3, 156.5, 150.6, 144.2, 144.0, 141.4, 138.2, 131.2, 130.9, 129.6, 127.8, 127.3, 126.5, 125.5, 125.1, 124.5, 120.0, 116.4, 105.4, 67.4, 55.2, 52.3, 47.3, 36.9; FAB-MS: calcd. $C_{31}H_{28}N_2O_5$ 508.55, found 508.2.

α-N-Fmoc-(αS)-β-(6'-Hydroxy-2'-naphthoyl)alanine (**4b**). R_f = 0.57; Yield: 0.86 g (89%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.02 (s, 1H), 7.91-7.72 (m, 4H), 7.55 (m, 2H), 7.42-7.23 (m, 6H), 7.09 (m, 1H), 7.06 (s, 1H), 4.94 (br, 1H), 4.65-4.46 (m, 3H), 3.28 (m, 1H), 3.12 (m, 1H); ^{13}C-NMR (106 MHz, $CDCl_3$) δ 165.6, 158.2, 156.5, 150.6, 145.1, 142.3, 138.2, 131.8, 130.6, 128.9, 127.0, 126.8, 126.5, 125.5, 118.4, 109.4, 66.8, 56.2, 50.9, 46.1; FAB-MS: calcd. $C_{29}H_{23}NO_6$ 481.5, found 482.3.

α-N-Fmoc-(αS)-β-[(4'-Hydroxy)benzoyl]alanine (**4c**). R_f = 0.55; Yield: 0.79 g (92%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.91-7.8 (m, 4H), 7.53 (s, 2H), 7.41-7.23 (m, 5H), 6.86 (m, 2H), 5.04 (br, 1H), 4.71-4.36 (m, 3H), 3.08 (m, 1H), 2.93 (m, 1H); ^{13}C-NMR (106 MHz, $CDCl_3$) δ 175.6, 168.2, 166.5, 158.6, 144.2, 142.7, 130.8, 128.9, 128.3, 126.8, 116.4, 66.8, 55.2, 51.4, 44.9; FAB-MS: calcd. $C_{25}H_{21}NO_6$ 431.44, found 432.5.

α-N-Fmoc-(αS)-β-[(4'-Dimethylamino)benzoyl]alanine (**4d**). R_f = 0.60; Yield: 0.81 g (88%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.88-7.81 (m, 4H), 7.57 (m, 2H), 7.45-7.12 (m, 5H), 6.94 (s, 2H), 4.96 (br, 1H), 4.42-4.08 (m, 3H), 3.18 (m, 1H), 2.89 (m, 1H), 3.02 (s, 6H); ^{13}C-NMR (106 MHz, $CDCl_3$) δ 173.3, 168.5, 162.6, 154.2, 142.8, 141.4, 129.7, 128.6, 126.5, 126.2, 114.45, 68.2, 55.6, 50.7, 46.3, 38.9; FAB-MS: calcd. $C_{27}H_{26}N_2O_5$ 458.51, found 458.6.

Typical experimental procedure for the synthesis of N-acetylated aminoamides

N-acetylated and carboxamidated Ald, Alb and other two amino acids were synthesized on a TentaGel S RAM resin (Sigma-Aldrich) respectively. After removal of the Fmoc group on the resins, the *N*-Fmoc-protected amino acids were attached to the resin respectively by using PyBOP/HOBt as coupling reagents. Fmoc deprotection, acetylation and cleavage from the resin by TFA/anisole treatment yielded the title compounds. Crude residues were purified by preparative reversed-phase HPLC using a linear gradient of 20-65% MeOH in 0.1% TFA over 25 min, respectively.

N-Ac-(αS)-β-[6'-(N, N-Dimethyl)amino-2'-naphthoyl]alanyl amide (**5a**). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.43 (s, 1H), 8.06 (s, 1H), 7.9 (d, 9.2, 1H), 7.8 (d, 7.1, 1H), 7.66 (d, 8.8, 1H), 7.23 (s, 2H), 7.06 (d, 7.9, 1H), 6.94 (s, 1H), 4.70 (m, 1H), 3.25 (m, 1H), 2.93 (m, 1H), 3.05 (s, 6H), 1.79 (s, 3H); ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ 196.6, 174.0, 169.9, 150.9, 137.9, 131.4, 130.3, 129.5, 126.6, 125.2, 124.5, 117.1, 105.4, 49.7, 40.8, 39.5, 23.2; FAB-MS *m/z*: calcd. for $C_{18}H_{22}N_3O_3$ ($[M+H]^+$) 328.16; found 328.13.

N-Ac-(αS)-β-(6'-Hydroxy-2'-naphthoyl)alanyl amide (**5b**). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H), 8.53 (s, 1H), 8.24 (s, 1H), 8.09 (d, 8.5, 1H), 7.92 (d, 6.9, 1H), 7.83 (d, 8.5, 1H), 7.26 (s, 2H), 7.09 (s, 1H), 7.06 (s, 1H), 5.11 (m, 1H), 3.25 (m, 1H), 2.96 (m, 1H), 1.86 (s, 3H); ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ 196.6, 174.0, 169.9, 159.9, 134.5, 131.6, 131.4, 130.7, 127.7, 126.2, 125.5, 118.6, 108.4, 50.7, 45.8, 23.2; FAB-MS *m/z*: calcd. for $C_{16}H_{17}N_2O_4$ ($[M+H]^+$) 301.31; found 301.33.

N-Ac-(αS)-β-[(4'-Hydroxy)benzoyl]alanyl amide (**5c**). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 8.42 (s, 1H), 7.91 (d, 9.0, 2H), 7.24 (s, 2H), 6.78 (d, 9.0, 2H), 4.86 (m, 1H), 3.62 (m, 2H), 1.96 (s, 3H); ^{13}C- NMR (100.6 MHz, DMSO-d_6) δ 192.8, 174.2, 170.1, 162.6, 130.4, 129.5, 117.3, 51.5, 45.6, 22.8; FAB-MS *m/z*: calcd. for $C_{12}H_{14}N_2O_4$ ($[M+H]^+$) 251.25; found 251.23.

N-Ac-(αS)-β-[(4'-dimethylamino)benzoyl]alanyl amide (**5d**). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 1H), 7.86 (d, 9.0, 2H), 7.21 (s, 2H), 6.89 (d, 9.0, 2H), 4.36 (m, 1H), 3.52 (m, 2H), 3.11 (s, 6H), 1.86 (s, 3H); ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ 192.8, 174.2, 170.1, 154.3, 129.9, 125.2, 114.3, 51.2, 44.6, 39.7, 22.8; FAB-MS *m/z*: calcd. for $C_{14}H_{20}N_3O_3$ ($[M+H]^+$) 278.14; found 278.11.

Acknowledgements

The authors would like to thank the financial support from the Accented Project for Natural Scientific Research of Universities in Guangdong Province (No. 132112) and National Scientific

Foundation of China (No. 30672560). The author would like to express heartfelt thanks to Prof. Dr. Peter W. Schiller for his kindness and very useful advice in this work. Thanks are also due to Prof. Dr. Bogumil Zelent for his help in part of fluorescence determinations.

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Sample Availability: Contact the authors.