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Synthesis of Enantiopure Fmoc-α-Methylvaline

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Abstract:

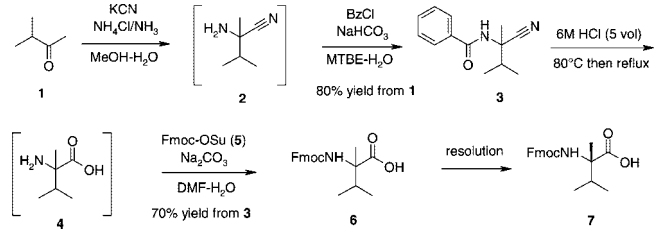
An efficient synthesis of enantiopure Fmoc-α-methylvaline has been developed. The racemate was prepared in two steps from 3-methyl-2-butanone and was resolved using a chiral amine, (*S*)-1,2,3,4-tetrahydro-1-naphthylamine to give the desired, enantiopure *S*-isomer in 23% overall yield.

Introduction

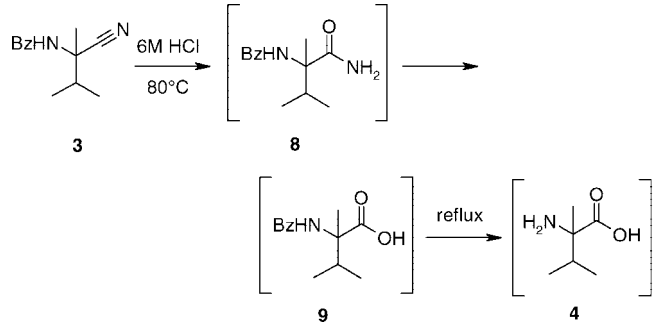
A recent program at Roche required kilogram quantities of an unnatural amino acid building block, Fmoc-α-methylvaline (**7**). While α-methylvaline and the corresponding Fmoc derivative are both commercially available from a number of suppliers, they are quite expensive, and none of the vendors was able to produce the required quantities of this material within the required time frame. Asymmetric syntheses of α-methylvaline using chiral auxiliaries or chiral phase-transfer catalysis have been reported by Seebach and other groups.[1] Spino et al. described a stereodivergent approach to chiral N-protected α,α-dialkylated amino acids starting from *p*-menthane-3-carboxaldehyde.[2] Enzymatic resolutions of racemic α-methylvaline derivatives have also been reported.[3] However, none of these methods are considered adequate for large scale preparation in terms of cost and scalability. More recently, during the preparation of this manuscript, an elegant preparation of N-ethoxycarbonyl-α-methylvaline (91–95% ee) using a dynamic resolution appeared.[4] Herein we describe a practical synthesis of enantiopure (>99% ee) Fmoc-α-methylvaline (**7**).

Results and Discussion

Preparation of Racemic Fmoc-α-methylvaline. Racemic α-methylvaline was prepared *via* a Strecker reaction followed by benzoyl group assisted hydrolysis of the nitrile group.[5] Thus, 3-methyl-2-butanone (**1**) was added to a mixture of potassium cyanide, ammonium hydroxide and ammonium chloride in methanol (1 vol.). After stirring at room temperature overnight, water (3 vol.) was added to dissolve the inorganic salts and product **2** was extracted with methyl *tert*-butyl ether (MTBE). The resulting solution of **2** was washed with water, partially concentrated to remove residual ammonia, and treated with benzoyl chloride in the presence of aqueous sodium bicarbonate. After stirring at room temperature overnight, the resulting solid was collected by filtration to give benzamide **3** as white solid in 80% overall yield from **1** and 100% purity as determined by HPLC analysis.



The direct hydrolysis of **2**, a relatively volatile oil, to acid **4** was not investigated since such a hydrolysis usually requires quite harsh conditions.[4] On the other hand, the benzoyl group in **3** was expected to facilitate the hydrolysis of the nitrile group through neighboring group participation[5] and also ease reaction monitoring by HPLC. Thus, a mixture of **3** and 6 M hydrochloric acid (5 vol.) was heated to 80 °C for 2–3 h to cleanly give acid **9** via amide **8** as indicated by HPLC analysis, then the reaction mixture was heated to reflux for 8 h to remove the benzoyl group to give acid **4**. The aqueous reaction mixture was washed twice with MTBE to remove the resulting benzoic acid and basified to pH ∼10.5 by the addition of 50% sodium hydroxide. Then ammonia was removed by partial concentration under reduced pressure. The resulting aqueous solution of **4** was used directly in the next step.



The aqueous solution of **4** prepared above was diluted with DMF, then a solution of Fmoc-OSu (0.95 equiv in DMF) was added dropwise over 2–3 h at room temperature, while maintaining the mixture at pH 9–10 with the addition of 20% sodium carbonate solution. After stirring for an additional 30 min, the insolubles were removed by filtration. The filtrate was then extracted twice with MTBE to remove unreacted Fmoc-OSu and the byproduct, 9-fluorenemethanol and 9-methylenefluorene. The aqueous phase was acidified with concentrated

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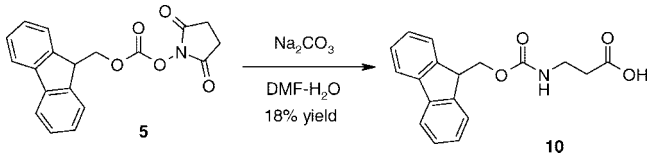
Table 1. **Resolution of 6 with (*S*)-1,2,3,4-tetrahydro-1-naphthylamine (11)**

entry	scale **6** (g)	amine **11** (equiv)	solvent (vol)	salt **12** (*S:R*)	filtrate (*S:R*)	yield (%)
1	0.1	1	EtOAc (20)	98:2	18:82	33
2	0.1	1	MeOH (20)		no crystallization observed	
3	0.1	1	EtOH (20)		no crystallization observed	
4	0.1	1	iPrOAc (20)	97:3	12:88	nd
5	0.1	1	MTBE (20)	94:6	15:85	44
6	1.0	1	iPrOAc (20)	96:4	12:88	44
7	9.5	1	iPrOAc (14)	94:6	9:91	47
8	0.5	0.75	iPrOAc (14)	96:4	9:91	42
9	0.5	0.5	iPrOAc (14)	98:2	24:76	32
10	5.9	1	iPrOAc (14)	96:4	12:88	44

hydrochloric acid to pH 2–3, and extracted with isopropyl acetate. The extract was washed twice with water to remove DMF, and then partially concentrated. Racemate **6** crystallized upon slow addition of heptane and was isolated in 70% yield and >99% purity.

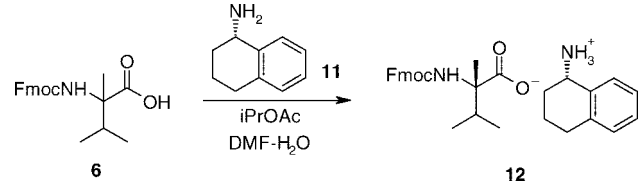
A major impurity in racemate **6** was identified as Fmoc-β-alanine (**10**). Fmoc-β-alanine and its derivatives are common contaminants found in commercial Fmoc-amino acids.[6] In a controlled experiment, **10** was obtained in 18% isolated yield in the absence of amino acid, indicating it was generated by the decomposition of Fmoc-OSu (**5**). This is in agreement with the pathway proposed by Isidro-Llobet et al. recently.[7] Lossen rearrangement of closely related compounds has also been described by other groups.[8]



In order to minimize the formation of **10** during the preparation of **6**, excess amount of Fmoc-OSu (**5**) should be avoided. With substoichiometric amount (i.e., 0.95 equiv) of **5** added dropwise as a DMF solution to **4**, the formation of **10** was well controlled to less than 2 area % in the reaction mixture. After crystallization, residual level of **10** dropped to ~0.2 area % in the product **6**. On the contrary, when **5** was added portionwise as solid, the reaction was slower, and a higher level of **10** (up to 9%) formed.

Resolution of Racemic Fmoc-α-methylvaline. The resolution of racemate **6** was initially attempted in ethanol or ethyl acetate with ten different chiral amines: α-methylbenzylamine, 1-(4-bromophenyl)ethylamine, cinchonine, 1-cyclohexylamine, 1-(2-naphthyl)ethylamine, *N*-benzylmethylbenzylamine, 1,2,3,4-tetrahydro-1-naphthylamine, (-)-sparteine, quinidine, and brucine. Only (*S*)-1,2,3,4-tetrahydro-1-naphthylamine (**11**) formed a crystalline salt with the desired *S*-enantiomer. Thus, the resolution using **11** was further investigated, and the results are summarized in Table 1. On the basis of this study, isopropyl acetate was selected as the preferred solvent for the salt formation.

The resolution was thus carried out with 0.8 equiv of **11** in 15 volumes of isopropyl acetate at room temperature for 21 h. After filtration, salt **12** was obtained in 49% yield on 50 g scale and with an *S/R* ratio of 91.8:8.2; impurity **10** mostly stayed in the mother liquor. The chiral purity and chemical purity can be further upgraded to greater than 99.5% and 99.9%, respectively, in 82% recovered yield by recrystallization from aqueous DMF at room temperature. Trituration in isopropyl acetate at reflux was not successful due to significant deprotection (50% after 8 h) at this temperature.



Enantiopure (*S*)-Fmoc-α-methylvaline (**7**) can be generated from the resolved crystalline salt **12** by treatment of a suspension of **12** in ethyl acetate or MTBE with 1 M hydrochloric acid. However, since **7** can only be isolated as a white foam or viscous syrup, it is advantageous to store this material as its salt and convert it to **7** immediately prior to use.

Conclusion

In summary, we have developed an efficient synthesis of enantiopure Fmoc-α-methylvaline in three steps and 23% yield from 3-methyl-2-butanone. The process has been scaled up to produce kilogram quantities of this material to support our drug substance supply campaigns.

Experimental Section

Caution: Potassium cyanide is highly toxic. Care should be taken when handling this material.[9]

***N*-(1-Cyano-1,2-dimethylpropyl)benzamide (3).** To a stirred mixture of potassium cyanide (94.51 g, 1.45 mol), ammonium hydroxide (225.7 mL, 3.48 mol), and ammonium chloride

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(77.63 g, 1.45 mol) in methanol (100 mL) was added 3-methyl-2-butanone (100 g, 1.16 mol). The mixture was stirred at room temperature overnight, then water (300 mL) was added to dissolve the inorganic salts. The resulting solution was extracted with MTBE (3 × 300 mL). The combined extracts (~1050 mL) were washed with water (150 mL), then concentrated under reduced pressure to remove ~450 mL of solvent. To the remaining solution was added MTBE (200 mL), water (600 mL), and sodium bicarbonate (146.3 g, 1.74 mol). The mixture was cooled with an ice–water bath, then benzoyl chloride (149.7 mL, 1.28 mol) was added dropwise over 1 h. After the addition was complete, the resulting slurry was stirred at room temperature overnight; HPLC analysis indicated complete reaction. The solid was then collected by filtration, washed with water (600 mL) and MTBE−heptane (1:1, 600 mL), and dried in a vacuum oven at 55 °C overnight to give the product **3** (201.0 g, 80% yield) as a white solid. The product purity was 100% as determined by HPLC analysis (UV 254 nm). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56 (s, 1H), 7.81 (m, 2H), 7.56 (m, 1H), 7.47 (m, 2H), 2.49 (m, 1H), 1.54 (s, 3H), 1.07 (d, J = 6.6 Hz, 3H), 0.93 (d, J = 6.6 Hz, 3H).

Racemic *N*-Fmoc-α-methylvaline (6). A slurry of **3** (40.0 g, 184.9 mmol) in concentrated hydrochloric acid (100 mL, 1.2 mol) and water (100 mL) was stirred at 80 °C for 2.5 h, then at reflux for 8 h. The mixture was cooled to room temperature and extracted with MTBE (2 × 160 mL). The aqueous layer was then cooled with an ice–water bath, and basified with sodium hydroxide (50%, 70 mL, 1.34 mmol) to pH ~10.5. The resulting solution (~320 mL) was concentrated at 50 °C and 40 mmHg to ~160 mL, and then diluted with water (80 mL) and DMF (160 mL). To the resulting mixture was simultaneously added dropwise a solution of Fmoc-OSu (59.24 g, 175.7 mmol) in DMF (160 mL) and 20% sodium carbonate (170 mL, 378.5 mmol) over 2.5 h. The pH of the reaction mixture was continuously monitored by a pH meter and was maintained between 9.5 and 10 by adjusting the addition rate of sodium carbonate. After the addition was complete, the mixture was stirred for an additional 30 min and then filtered to remove insolubles. The filtrate was washed with MTBE (2 × 240 mL), and the aqueous phase was acidified with concentrated hydrochloric acid (32 mL, 384 mmol) to pH ~2.5 and then extracted with isopropyl acetate (320 mL). The extract was washed with water (2 × 160 mL), then concentrated to ~120 mL. Heptane (480 mL) was added dropwise, and seed crystals were added when the solution turned cloudy. After stirring for an additional 1 h, the resulting solid was collected by filtration and washed with heptane (200 mL) and then dried by suction to give the product **6** (45.50 g, 70% yield) as a white solid. The product purity was 99.61% as determined by HPLC analysis (UV 254 nm). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.30 (s, 1H), 7.88 (d, J = 7.2 Hz, 2H), 7.72 (d, J = 7.2 Hz, 2H), 7.40 (m, 3H), 7.31 (m, 2H), 4.19 (m, 3H), 2.01 (m, 1H), 1.27 (s, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.84 (d, J = 6.6 Hz, 3H).

Fmoc-α-methylvaline Salt (12). To a stirred solution of **6** (50.0 g, 141.5 mmol) in isopropyl acetate (750 mL) was added (*S*)-1,2,3,4-tetrahydro-1-naphthylamine (16.83 g, 113.2 mmol) dropwise. The mixture was stirred at room temperature for 21 h. The resulting solid was collected by filtration, washed with isopropyl acetate (3 × 40 mL), and dried by suction to give the salt **12** (34.80 g, 49% yield) as a white solid. The chemical purity of the product was 99.96% and the dr was 91.8:8.2 as determined by HPLC analysis.

To a solution of **12** (34.0 g, 67.91 mmol) obtained above in DMF (272 mL) was added water (68 mL) dropwise while maintaining the internal temperature below 35 °C to initiate crystallization. The mixture was stirred for 10 min, then additional water (102 mL) was added dropwise while maintaining the temperature between 30 and 35 °C. The resulting slurry was cooled to room temperature over 1 h, then stirred at this temperature for an additional 30 min. The resulting solid was collected by filtration, washed with DMF−water (1:1, 136 mL) and water (2 × 68 mL), and dried by suction to give the product **12** (28.0 g, 82% recovery). The chemical purity of the product was 99.98% and the dr was 99.78:0.22 as determined by HPLC analysis (UV 254 nm). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.87 (d, J = 7.2 Hz, 2H), 7.65 (d, J = 7.2 Hz, 2H), 7.08–7.47 (m, 9H), 4.10–4.30 (m, 4H), 3.33 (br, 3H), 2.70 (m, 2H), 2.10 (m, 1H), 1.72–2.03 (m, 2H), 1.60–1.72 (m, 2H), 1.32 (s, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.78 (d, J = 6.6 Hz, 3H).

Formation of Fmoc-β-alanine (10) from Fmoc-OSu (5). To a solution of sodium carbonate (4.71 g, 44.5 mmol) in water (50 mL) and DMF (50 mL) was added **5** (10.0 g, 29.6 mmol) portionwise over 30 min. The mixture was stirred at room temperature overnight, then the insolubles were removed by filtration. The filtrate was washed with MTBE (3 × 50 mL), acidified to pH ~3, and extracted with ethyl acetate (100 mL). The extract was washed with water (3 × 50 mL), and concentrated to ~25 mL. Then heptane (50 mL) was added dropwise. The product was collected by filtration and washed with heptane to give **10** (1.7 g, 18% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.21 (s, 1H), 7.87 (d, J = 7.5 Hz, 2H), 7.67 (d, J = 7.5 Hz, 2H), 7.25–7.45 (m, 5H), 4.27 (d, J = 6.6 Hz, 2H), 4.19 (t, J = 6.6 Hz, 1H), 3.18 (m, 2H), 2.37 (t, J = 7.2 Hz, 2H).

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