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Tetrahedron: *Asymmetry* 15 (2004) 1181–1186
Tetrahedron: *Asymmetry*

Asymmetric synthesis of N,O-diprotected (2*S*,3*S*)-*N*-methyl-δ-hydroxyisoleucine, noncoded amino acid of halipeptin A

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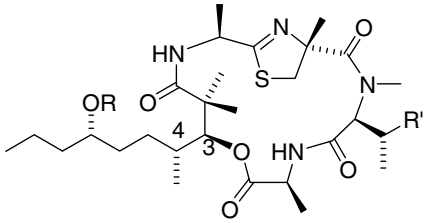
Received 2 February 2004; accepted 5 February 2004

Dedicated to Prof. Adolfo Zambelli on the occasion of his 70th birthday

Abstract—The first enantioselective synthesis of N,O-diprotected (2*S*,3*S*)-*N*-methyl-δ-hydroxyisoleucine **4**, starting from readily available (*tert*-butyldimethylsilyl)-2-butyn-1-ol **5**, is described. The key steps of this stereochemical flexible synthetic route involve a silyl-assisted [3,3]-sigmatropic rearrangement, for the establishment of the correct stereoisomeric pattern, and a triethylsilane–TFA induced reduction of an oxazolidinone intermediate, to yield the requested N-methylation.

1. Introduction

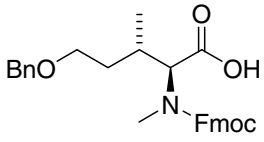
N-Alkyl amino acids are quite common in bioactive natural peptides[1] and show important influence on membrane permeability, proteolitic stability and conformational rigidity.[2] Two of these *N*-methyl-δ-hydroxyisoleucine and *N*-methyl-L-valine have recently been found in the naturally occurring potent anti-inflammatory cyclic depsipeptides halipeptin A[3] **1**, B[3] **2** and C[4] **3**, where they contribute to the 17-membered macrolactone ring.



1 halipeptin A, R = Me, R' = CH_2CH_2OH
2 halipeptin B, R = H, R' = CH_2CH_2OH
3, halipeptin C, R = H, R' = CH_3

The C-2/C-3 relative configuration of the *N*-methyl-δ-hydroxyisoleucine was established to be *erythro* [(2*R*,3*R*) or (2*S*,3*S*)] by applying the *J*-based Murata's method.[3] The absolute configurations were then assigned as (2*S*,3*S*) considering that halipeptin C **3** contained an *N*-methyl-valine belonging to the L-series [(*S*)-configuration, Marfey analysis][4] and that the ^{1}H NMR of halipeptin B[3] **2** and C[4] **3** were 'virtually superimposable, except for few signal'.[4]

As a part of our investigation towards halipeptin A total synthesis and with the aim of developing a stereochemically flexible synthetic route useful for the elaboration of a solid-phase compatible, protected version of *N*-methyl-δ-hydroxyisoleucine, herein we report the first[5] asymmetric synthesis of the N,O-diprotected (2*S*,3*S*)-*N*-methyl-δ-hydroxyisoleucine **4**.



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2. Results and discussion

Our approach to the key intermediate (2*S*,3*S*)-2-amino-3-methyl-4-pentenoic acid **11** was realized through a silyl-assisted [3,3]-sigmatropic rearrangement[6] of the

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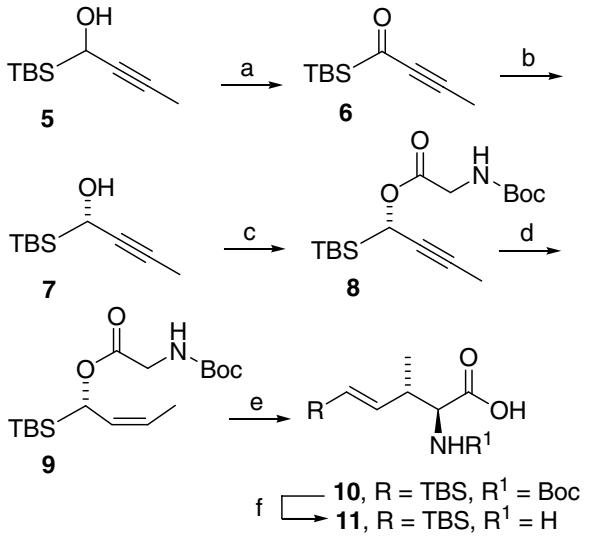
doi:10.1016/j.tetasy.2004.02.008

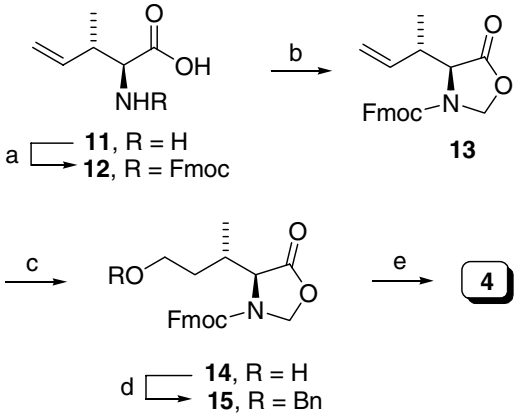
optically active (*S*)-(*Z*)-1-(*tert*-butyldimethylsilyl)-2-butenyl *N*-*tert*-butyloxycarbonylglycinate **9** (Scheme 1). This last compound was prepared starting from the readily available chiral nonracemic (*S*)-alcohol **7** (ee >95%)[7] through esterification with commercially available *N*-Boc-glycine and a subsequent stereoselective reduction of the alkyne moiety. The chelate–enolate Claisen rearrangement was performed under Kazmaier's conditions[8] and gave the expected (*E*)-*anti*-vinylsilane **10** in quantitative yield and with complete chirality transfer.[9] Quantitative removal of both *tert*-butyldimethylsilyl and *tert*-butyloxycarbonyl groups was possible using an excess of HBF_4 (80.0 equiv). Smaller amounts of HBF_4, even for prolonged reaction times at higher temperatures, induced N-deprotection and only partial desilylation.



Scheme 1. Reagents and conditions: (a) PDC, 4 Å m.s., CH_2Cl_2, 3 h, 84%; (b) (*S*)-Me-CBS reagent, BH_3·THF, THF, 30 min, 94%, ee >95%; (c) *N*-Boc-glycine, EDC, DMAP, CH_2Cl_2, 2 h; (d) Pd/5% wt. on $CaCO_3$, H_2, 3 h, 45 min, 75% (two steps); (e) LDA, $ZnCl_2$, THF, −78 °C, 1 h and 30 min (quant.); (f) HBF_4 (48% in water), 1,4-dioxane, 70 °C, 22 h, 75%.

Attention was then turned towards N-methylation and regioisomeric hydration of the terminal double bond. There are a number of methods useful for preparing *N*-methyl amino acids; however the basic conditions often necessary would induce partial racemization.[10] Freidinger et al.[11] reported a method in which *N*-Fmoc amino acids were reacted with paraformaldehyde in the presence of catalytic amounts of *p*-TsOH to form oxazolidinones. These can be subsequently reduced to N-methylated amino acids once treated with a few equivalents of triethylsilane, using trifluoroacetic acid as co-solvent. A unified approach to the synthesis of *N*-methyl derivatives of the 20 common L-amino acids, through 5-oxazolidinones, has since been reported by Aurelio et al.[12] showing the reliability of this synthetic methodology.

We thus converted amino acid **11** to *N*-Fmoc-protected 5-oxazolidinone **13** (Scheme 2) by treatment with 9-fluorenylmethyl chloroformate, followed by cyclization with paraformaldehyde.[13] Side-chain functionalization proved quite difficult to achieve because of the inherently unstable protected heterocycle **13**.[14] A satisfying hydroboration–oxidation reaction was eventually obtained using BH_3·SMe_2 (3 equiv) at 0 °C followed by the use of the mild oxidation reagent sodium perborate,[15] compatible with the base labile fluorenylmethoxycarbonyl functionality. Under these conditions, primary alcohol **14** was obtained in 55% yield (based on the recovered starting material) with no evidence of regioisomeric secondary alcohol formation. Protection of the hydroxyl group was then attempted with TBS-Cl, (1.0–4.0 equiv) in the presence of triethylamine (1.0–5.0 equiv) in CH_2Cl_2 at 0 °C or rt; or with acetic anhydride (2.0 equiv)/pyridine (2.1 equiv) in CH_2Cl_2 at 0 °C or rt. In both cases the reactions proceeded very slowly and resulted in partial loss of the Fmoc protecting group or complete decomposition of the starting material. Successful protection of the hydroxy group was finally achieved with benzyl bromide in the presence of Ag_2O.[16] The etherification was quenched after 3 days and furnished the desired product **15** in 45% yield, based on the recovered starting material (36%). Compound **12** was then submitted to the reduction step to cleave the endocyclic C–O bond and liberate the *N*-methyl group. Unfortunately the mixture $Et_3SiH/CF_3CO_2H/CHCl_3$ proved quite aggressive, giving the target compound **4** in modest yield (21%).[17]



Scheme 2. Reagents and conditions: (a) Fmoc-Cl, Na_2CO_3, 1,4-dioxane, 0 °C, 18 h, 76%; (b) $(CH_2O)_n$, *p*-TsOH, toluene, reflux, 1 h, 62%; (c) BH_3·SMe_2, 0 °C, 5 h then, EtOH, H_2O, $NaBO_3$·$4H_2O$, rt, 3 h, 55%; (d) BnBr, Ag_2O, CH_2Cl_2, 3 days, 45%; (e) Et_3SiH, TFA/$CHCl_3$, rt, 3 days, 21%.

3. Conclusion

In summary, we have accomplished the first asymmetric synthesis of the N,O-diprotected (2*S*,3*S*)-*N*-methyl-δ-hydroxyisoleucine **4** through a stereospecific chelate–enolate Claisen rearrangement as key step. Further studies to improve the yield of the triethylsilane–TFA induced reduction on labile oxazolidinone intermediate

and towards the total synthesis of **1** are currently underway.

4. Experimental

All reactions were carried out under a dry argon atmosphere using freshly distilled solvents, unless otherwise noted. Tetrahydrofuran (THF) was distilled from $LiAlH_4$. Toluene and methylene chloride were distilled from calcium hydride. Glassware was flame-dried (0.05 Torr) prior to use. When necessary, compounds were dried in vacuo over P_2O_5 or by azeotropic removal of water with toluene under reduced pressure. Starting materials and reagents purchased from commercial suppliers were generally used without purification. Reaction temperatures were measured externally; reactions were monitored by TLC on Merck silica gel plates (0.25 mm) and visualized by UV light, I_2, or spraying with $KMnO_4$, *p*-anisaldehyde or phosphomolybdic acid solutions and drying.

Flash chromatography was performed on Merck silica gel 60 (particle size: 0.040–0.063 mm). Yields refer to chromatographically and spectroscopically (^1H and ^{13}C NMR) pure materials. The NMR spectra were recorded at room temperature or when indicated at 80 or 100 °C on a Bruker DRX 400, a Bruker DRX 300 and a Bruker AM 250 spectrometers. Chemical shifts are reported relative to the residual solvent peak ($CHCl_3$: $\delta = 7.26$, $^{13}CDCl_3$: $\delta = 77.0$; $C_2H_2Cl_4$: $\delta = 5.80$, $^{13}C_2D_2Cl_4$: $\delta = 72.1$; HOD: $\delta = 4.79$). High resolution spectra (EI- and ES-MS) were performed on a VG 70-250SE and a Q-Star Applied Biosystem mass spectrometers. Optical rotations were measured with a JASCO DIP-1000 polarimeter.

4.1. 1-*tert*-Butyldimethylsilyl)-2-butyn-1-ol, 5

To a solution of 2-butyn-1-ol (2.53 g, 42.8 mmol) in dry THF (45.0 mL), *n*-BuLi (2.5 M in hexane, 18.0 mL, 44.9 mmol) was added at −78 °C. The mixture was stirred at 0 °C for 30 min. To the mixture was added a solution of TBS-Cl (6.77 g, 44.9 mmol) in THF (9.0 mL) at −78 °C. After stirring at room temperature for 16 h, *n*-BuLi (2.5 M in hexane, 20.5 mL, 51.4 mmol) was added and the reaction mixture stirred at −45 °C for 3 h. The reaction was then quenched by addition of 10% AcOH in THF at −78 °C and extracted with diethyl ether. The organic phase was washed with saturated $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash-chromatography (5–10% diethyl ether in petroleum ether) to afford **5** (6.62 g, 84%) as a pale yellow oil. $R_f = 0.74$ (30% diethyl ether in petroleum ether). ^1H NMR ($CDCl_3$, 400 MHz): δ 0.07 (3H, s, (CH_3)–Si), 0.10 (3H, s, (CH_3)–Si), 0.97 (9H, s, –((CH_3)$_3$–C)Si), 1.87 (3H, d, $J = 2.6$ Hz, H-4), 4.18 (1H, q, $J = 2.6$ Hz, H-1). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −8.6, −8.0. 3.8, 16.9, 26.8 (×3), 55.0, 80.1, 84.0 HR-EIMS m/z 184.1287 (calcd 184.1283 for $C_{10}H_{20}OSi$).

4.2. 1-(*tert*-Butyldimethylsilyl)-2-butyn-1-one, 6

To a solution of 1-(*tert*-butyldimethylsilyl)-2-butyn-1-ol (7.33 g, 39.8 mmol) in dry CH_2Cl_2 (300 mL), 4 Å molecular sieves (14.7 g) and PDC (24.1 g, 64.1 mmol) were added. The mixture was stirred at room temperature for 3 h, then diluted with diethyl ether (300 mL) and allowed to stir for an additional 30 min. Filtration through a short pad of silica gel (particle size 0.063–0.200 mm) and $CaSO_4$ (10% in weight) afforded a solution, which was concentrated in vacuo. The residue was flash-chromatographed (25% diethyl ether in petroleum ether) to afford **6** (6.10 g, 84%) as a pale yellow oil. $R_f = 0.67$ (30% diethyl ether in petroleum ether). ^1H NMR ($CDCl_3$, 400 MHz): δ 0.22 (6H, s, (CH_3)$_2$–Si), 0.97 (9H, s, –((CH_3)$_3$–C)Si), 2.09 (3H, s, H-4). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −7.5 (×2), 4.5, 16.9, 26.9 (×3), 85.0, 98.5, 177.2. HR-EIMS m/z 182.1120 (calcd 182.1127 for $C_{10}H_{18}OSi$).

4.3. (*S*)-1-(*tert*-Butyldimethylsilyl)-2-butyn-1-ol, 7

(*S*)-Me-CBS reagent (1 M in toluene, 11.0 mL, 11.0 mmol) and BH_3·THF (1 M in THF, 27.4 mL, 27.4 mmol) were mixed, under N_2 and at −78 °C, and allowed to stir for 30 min. A solution of 1-(*tert*-butyldimethylsilyl)-2-butyn-1-one (1.00 g, 5.48 mmol) dissolved in dry THF (18.0 mL) was then added via cannula. The reaction mixture was stirred for 30 min. Methanol (17.0 mL) was added and the solution allowed to stir for an additional 30 min at −78 °C, diluted with diethyl ether and allowed to warm to room temperature. The mixture was washed with 5% solution of $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was flash-chromatographed (20% diethyl ether in petroleum ether) to give **7** (0.95 g; 94%, ee >95%). $R_f = 0.74$ (30% diethyl ether in petroleum ether). ^1H NMR ($CDCl_3$, 400 MHz): δ 0.07 (3H, s, (CH_3)–Si), 0.10 (3H, s, (CH_3)–Si), 0.97 (9H, s, –((CH_3)$_3$–C)Si), 1.87 (3H, d, $J = 2.6$ Hz, H-4), 4.18 (1H, q, $J = 2.6$ Hz, H-1). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −8.6, −8.0, 3.8, 16.9, 26.8 (×3), 55.0, 80.1, 84.0. HR-EIMS m/z 184.1285 (calcd 184.1283 for $C_{10}H_{20}OSi$). $[\alpha]_D^{25} = -73.8$ (c 1.2, $CHCl_3$).

4.4. (*S*)-1-(*tert*-Butyldimethylsilyl)-2-butynyl *N*-*tert*-butyloxycarbonyl-glycinate, 8

To a solution of (*S*)-1-(*tert*-butyldimethylsilyl)-2-butyn-1-ol (1.33 g, 7.22 mmol) in dry CH_2Cl_2 (23.0 mL) *N*-Boc-glycine (3.86 g, 22.0 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC, 2.31 g, 12.1 mmol) and *N*,*N*-dimethylaminopyridine (DMAP, 0.044 g, 0.360 mmol) were added at 0 °C. The reaction mixture was stirred at 0 °C for 2 h, then concentrated in vacuo, diluted with diethyl ether and washed with saturated aq $NaHCO_3$ and brine. The combined organic phases were dried over $MgSO_4$, filtered and concentrated in vacuo. Crude **8** was used in the next step without further purification. $R_f = 0.31$ (20% diethyl ether in petroleum ether). ^1H NMR ($CDCl_3$, 400 MHz):

δ 0.09 (3H, s, (CH_3)–Si), 0.10 (3H, s, (CH_3)–Si), 0.94 (9H, s, –((CH_3)$_3$–C)Si), 1.45 (9H, s, –((CH_3)$_3$–CO) 1.85 (3H, d, $J = 2.6$ Hz, H-4), 3.93 (2H, m, CO_2CH_2NH), 5.00 (1H, br s, NH), 5.29 (1H, m, H-1). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −8.1, −7.7, 3.9, 16.9, 26.7 (×3), 28.3 (×3), 42.6, 58.4, 75.9, 80.1, 84.6, 155.5, 170.1. HR-EIMS m/z 341.2026 (calcd 341.2022 for $C_{17}H_{31}NO_4Si$). $[\alpha]_D^{25} = -106.2$ (c 0.7, $CHCl_3$).

4.5. (*S*)-(*Z*)-1-(*tert*-Butyldimethylsilyl)-2-butenyl *N*-*tert*-butyloxycarbonylglycinate, 9

To a solution of crude **8** (1.22 g, 3.58 mmol) in ethyl acetate (9.0 mL), Lindlar's catalyst (Pd/5% wt. on $CaCO_3$, 0.764 g) was added and the mixture stirred under H_2 for 3.75 h. The suspension was filtered on a pad of Celite® and washed with CH_2Cl_2. Solvents were removed in vacuo and the residue flash-chromatographed (10–20% diethyl ether in petroleum ether) to give **9** (1.86 g, 75% for two steps). $R_f = 0.64$ (30% diethyl ether in petroleum ether). ^{1}H NMR ($CDCl_3$, 400 MHz): δ −0.02 (3H, s, (CH_3)–Si), 0.04 (3H, s, (CH_3)–Si), 0.91 (9H, s, –((CH_3)$_3$–C)Si), 1.44 (9H, s, –((CH_3)$_3$–CO), 1.71 (3H, dd, $J = 6.9$, 1.5 Hz, H-4), 3.88 (2H, m, CO_2CH_2NH), 4.99 (1H, br s, NH), 5.39 (1H, dd, $J = 10.7$, 10.4 Hz, H-2), 5.53, (1H, m, H-3), 5.65 (1H, br d, $J = 10.4$ Hz, H-1). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −8.2, −7.7, 13.6, 16.9, 26.8 (×3), 28.3 (×3), 42.7, 65.7, 79.8, 125.8, 127.2, 155.5, 170.2. HR-EIMS m/z 343.2175 (calcd 343.2179 for $C_{17}H_{33}NO_4Si$). $[\alpha]_D^{25} = -9.2$ (c 1.0, $CHCl_3$).

4.6. (2*S*,3*S*)-(*E*)-2-*tert*-Butoxycarbonylamino-5-(*tert*-butyldimethylsilyl)-3-methyl-4-pentenoic acid, 10

To a solution of lithium diisopropylamide (LDA, 2 M, in eptane/THF/ethylbenzene, 5.45 mL, 10.9 mmol) were added a solution of **9** (0.931 g, 2.71 mmol) and $ZnCl_2$ (1 M in ethyl ether, 3.25 mL, 3.25 mmol) in THF (16.0 mL) at −78 °C. The resulting mixture was stirred at room temperature 1.5 h. The pH of the reaction mixture was adjusted to 1 with 1 M HCl and the mixture was extracted with diethyl ether. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give **10** (0.931 g, quant.). $R_f = 0.42$ (10% methanol in chloroform). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.01 (6H, s, (CH_3)$_2$–Si), 0.85 (9H, s, –((CH_3)$_3$–C)Si), 1.12 (3H, d, $J = 6.8$ Hz, CH_3–), 1.44 (9H, s, –((CH_3)$_3$–CO), 2.80 (1H, m, H-3), 4.22 (1H, m, H-2), 4.89 (1H, br d, $J = 8.2$ Hz, NH), 5.77 (1H, d, $J = 18.6$ Hz, H-5), 5.87 (1H, dd, $J = 18.6$, 6.8 Hz, H-4). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −6.2 (×2), 16.2, 16.4, 26.4 (×3), 28.2 (×3), 42.5, 57.5, 80.2, 130.3, 146.5, 155.8, 176.5. HR-EIMS m/z 343.2171 (calcd 343.2179 for $C_{17}H_{33}NO_4Si$). $[\alpha]_D^{25} = +7.9$ (1.0, $CHCl_3$).

4.7. (2*S*,3*S*)-2-Amino-3-methyl-4-pentenoic acid, 11

To a solution of **10** (0.300 g, 0.874 mmol) in 1,4-dioxane (7.5 mL), HBF_4 (48% in water, 9.7 mL, 69.9 mmol) was added. The mixture was stirred for 22 h at 70 °C and allowed to reach room temperature. Saturated aq $NaHCO_3$ was then added and the mixture washed with diethyl ether. The pH of the mixture was adjusted to 1 with 1 M HCl, washed with diethyl ether and concentrated in vacuo. The residue was purified over Dowex 50 W (100–200 mesh, H^+ activated). H_2O followed by 1 M aqueous NH_3 eluted **11** (0.085 g; 75%). $R_f = 0.38$ (10% methanol in $CHCl_3$). ^{1}H NMR (D_2O, 250 MHz): δ 1.11 (3H, d, $J = 7.0$ Hz, –CH_3), 2.74 (1H, m, H-3), 3.53 (1H, d, $J = 5.6$ Hz, H-2), 5.19 (1H, d, $J = 10.0$ Hz, H-5), 5.20 (1H, d, $J = 17.1$ Hz, H′-5), 5.71 (1H, ddd, $J = 17.1$, 10.0, 7.7 Hz, H-4). ^{13}C NMR (D_2O, 1,4-dioxane, external standard: δ 66.7, 62.5 MHz): δ 15.5, 38.7, 59.3, 118.1, 137.0, 174.5. HR-ESMS m/z 130.0890 (calcd 130.0868 for $C_6H_{12}NO_2$). $[\alpha]_D^{25} = -18.0$ (c 1.1, H_2O).

4.8. (2*S*,3*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-4-pentenoic acid, 12

To a suspension of **11** (0.113 g, 0.874 mmol) in 1,4-dioxane (1.2 mL), Na_2CO_3 (10% in water, 2.6 mL) and a solution of 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl, 0.226 g, 0.874 mmol) in 1,4-dioxane (2.4 mL) were added at 0 °C. The resulting mixture was stirred for 18 h at room temperature, poured in water (45.0 mL) and extracted twice with diethyl ether to remove 9-fluorenylmethanol and dibenzofulvene. The pH of the aqueous phase was adjusted to 1 with 1 M HCl and the mixture extracted three times with ethyl acetate. The residue was flash-chromatographed (2–10% methanol in chloroform) to give **12** (0.233 g, 76%). $R_f = 0.5$ (10% methanol in chloroform). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.12 (3H, d, $J = 6.7$ Hz, –CH_3), 2.86 (1H, m, H-3), 4.22 (1H, t-like, $J = 7.0$ Hz, CH-Fmoc), 4.42 (3H, m, H-2 and CH_2-Fmoc overlapped), 5.09 (3H, m, H-5, H′-5 and NH overlapped), 5.63 (1H, ddd, $J = 16.0$, 11.0, 7.4 Hz, H-4), 7.31 (2H, t-like, $J = 7.3$ Hz, Ar), 7.39 (2H, t-like, $J = 7.3$ Hz, Ar), 7.58 (2H, d-like, $J = 6.8$ Hz, Ar), 7.76 (2H, d-like, $J = 6.8$ Hz, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 16.0, 39.7, 47.0, 58.0, 67.2, 117.3, 120.1 (×2), 125.0 (×2), 127.0 (×2), 127.7 (×2), 137.2, 141.2 (×2), 143.8 (×2), 156.4, 176.1. HR-ESMS m/z 352.1567 (calcd 352.1549 for $C_{21}H_{22}NO_4$). $[\alpha]_D^{25} = -3.8$ (c 1.0, $CHCl_3$).

4.9. (4*S*)-3-(9*H*-Fluoren-9-ylmethoxycarbonyl)-4-[(*S*)-3-buten-2-yl]-oxazolidin-5-one, 13

To a solution of **12** (0.172 g, 0.490 mmol) in dry toluene (11 mL), paraformaldehyde (0.011 g) and *p*-toluensulfonic acid (*p*-TsOH, 0.001 g) were added at 0 °C. The mixture was refluxed for 1 h with azeotropic water removal. The solution was cooled, washed with 1 M aqueous solution of $NaHCO_3$, extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was flash-chromatographed (10–20% ethyl acetate in petroleum ether) to give **13** (0.110 g, 62%). $R_f = 0.4$ (20% ethyl acetate in petroleum ether). ^{1}H NMR ($C_2D_2Cl_4$, 100 °C, 400 MHz): δ 0.81 (3H, d, $J = 6.7$ Hz, –CH_3), 2.54 (1H, m, –CHCH$_3$), 3.85 (1H, br s, H-4), 4.08 (1H, t-like, $J = 6.7$ Hz, CH-Fmoc), 4.53

(3H, d-like, $J = 6.7$ Hz, CH_2-Fmoc), 4.83, (1H, d, $J = 4.6$ Hz, H-2), 4.91 (1H, d, $J = 16.9$ Hz, –CH=C*H*H), 4.93 (1H, d, $J = 11.0$ Hz, –CH=CH*H*), 5.19 (1H, d, $J = 4.6$ Hz, H′-2), 5.55 (1H, ddd, $J = 16.9$, 11.0, 7.8 Hz, C*H*=CH_2), 7.18 (2H, t-like, $J = 7.3$ Hz, Ar), 7.26 (2H, t-like, $J = 7.3$ Hz, Ar), 7.39 (2H, d-like, $J = 6.8$ Hz, Ar), 7.61 (2H, d-like, $J = 6.8$ Hz, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 15.0, 40.1, 47.2, 59.6, 67.1, 78.2, 117.3, 120.1 (×2), 124.5 (×2), 127.2 (×2), 127.9 (×2), 137.0, 141.4 (×2), 143.2 (×2), 153.4, 171.0. HR-ESMS m/z 364.1571 (calcd 364.1549 for $C_{22}H_{22}NO_4$). $[\alpha]_D^{25} = +68.2$ (c 1.0, $CHCl_3$).

4.10. (4*S*)-3-(9*H*-Fluoren-9-ylmethoxycarbonyl)-4-[(*S*)-4-hydroxybutan-2-yl]-oxazolidin-5-one, 14

To a solution of **13** (0.071 g, 0.19 mmol) in dry THF (1.2 mL), $BH_3{\cdot}SMe_2$ (2 M in THF, 0.29 mL, 0.59 mmol) was added at 0 °C. The reaction mixture was stirred at 0 °C for 5 h and then EtOH (0.21 mL), H_2O (0.19 mL) and $NaBO_3{\cdot}4H_2O$ (0.09 g, 0.59 mmol) were added. The resulting mixture was stirred for an additional 3 h and then allowed to warm to room temperature. The mixture was concentrated in vacuo to remove any excess of THF, extracted with ethyl acetate and the combined organic phases dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was flash-chromatographed (25–40% ethyl acetate in petroleum ether) to give **14** (0.034 g, 55%, based on 15%—0.011 g—of recovered starting material **13**). $R_f = 0.5$ (50% ethyl acetate in petroleum ether). ^{1}H NMR ($C_2D_2Cl_4$, 100 °C, 300 MHz): δ 0.72 (3H, d, $J = 6.7$ Hz, –C*H*$_3$), 1.37 (1H, m, –C*H*HCH$_2$OH), 1.57 (1H, m, –CH*H*CH$_2$OH), 2.00 (1H, m, –C*H*CH$_3$), 3.40 (1H, m, –CH$_2$C*H*HOH), 3.47 (1H, m, –CH$_2$CH*H*OH), 3.91 (1H, d, $J = 4.0$ Hz, H-4), 4.09 (1H, t-like, $J = 6.7$ Hz, CH-Fmoc), 4.54 (3H, d-like, $J = 6.7$ Hz, CH_2-Fmoc), 4.86 (1H, d, $J = 4.6$ Hz, H-2), 5.16 (1H, d, $J = 4.6$ Hz, H′-2), 7.21 (2H, t-like, $J = 7.3$ Hz, Ar), 7.27 (2H, t-like, $J = 7.3$ Hz, Ar), 7.40 (2H, d-like, $J = 6.8$ Hz, Ar), 7.62 (2H, d-like, $J = 6.8$ Hz, Ar). ^{13}C NMR ($C_2D_2Cl_4$, 80 °C, 100 MHz): δ 12.4, 31.1, 33.2, 45.7, 56.8, 58.7, 65.5, 76.3, 118.3 (×2), 123.0 (×2), 125.5 (×2), 126.2 (×2), 139.6 (×2), 141.7 (×2), 151.2, 169.0. HR-ESMS m/z 382.1676 (calcd 382.1654 for $C_{22}H_{24}NO_5$). $[\alpha]_D^{25} = +14.8$ (c 1.0, $CHCl_3$).

4.11. (4*S*)-3-(9*H*-Fluoren-9-ylmethoxycarbonyl)-4-[(*S*)-4-benzyloxybutan-2-yl]-oxazolidin-5-one, 15

To a solution of **14** (0.026 g, 0.068 mmol) in dry CH_2Cl_2 (1.2 mL), Ag_2O (0.044 g, 0.19 mmol), $NaHCO_3$ (0.002 g) and BnBr (0.025 mL, 0.200 mmol) were added at 0 °C. The mixture was stirred in the dark for 5 h at room temperature. Ag_2O (0.044 g, 0.19 mmol) and BnBr (0.025 mL, 0.20 mmol) were again added. After 20 h at room temperature in the dark, the mixture was filtered and concentrated. The residue was flash-chromatographed (20–30% diethyl ether in petroleum ether) to afford **15** (0.009 g, 45%, based on 36%—0.009 g—of recovered starting material **13**). ^{1}H NMR ($C_2D_2Cl_4$, 80 °C, 400 MHz): δ 0.69 (3H, d, $J = 6.7$ Hz, –CH$_3$), 1.31 (1H, m, –C*H*HCH$_2$O–), 1.66 (1H, m, –CH*H*CH$_2$O–), 2.04 (1H, m, –C*H*CH$_3$), 3.28 (2H, m, –CH$_2$C*H*$_2$O–), 3.89 (1H, br s, H-4), 4.06 (1H, t-like, $J = 6.7$ Hz, CH-Fmoc), 4.27 (1H, d, $J = 12.0$ Hz, –C*H*HPh), 4.31 (1H, d, $J = 12.0$ Hz, –CH*H*Ph), 4.47 (3H, d-like, $J = 6.7$ Hz, CH_2-Fmoc), 4.87 (1H, d, $J = 4.6$ Hz, H-2), 5.18 (1H, d, $J = 4.6$ Hz, H′-2), 7.14 (7H, m, Ar-Fmoc and Ar–Bn overlapped), 7.24 (2H, t-like, $J = 7.3$ Hz, Ar-Fmoc), 7.37 (2H, d-like, $J = 6.8$ Hz, Ar-Fmoc), 7.59 (2H, d-like, $J = 6.8$ Hz, Ar-Fmoc). ^{13}C NMR ($C_2D_2Cl_4$, 80 °C, 100 MHz): δ 13.0, 30.2, 31.4, 45.7, 57.2, 65.6, 66.4, 71.0, 76.3, 118.3 (×2), 122.8 (×2), 125.5 (×2), 126.2 (×3), 126.5 (×2), 127.1 (×2), 136.9, 139.6 (×2), 141.7 (×2), 151.2, 169.0. HR-ESMS m/z 472.2146 (calcd 472.2124 for $C_{29}H_{30}NO_5$). $[\alpha]_D^{25} = +25.6$ (c 1.0, $CHCl_3$).

4.12. (2*S*,3*S*)-5-Benzyloxy-2-[(9*H*-fluoren-9-ylmethoxycarbonyl)-methylamino]-3-methyl-pentanoic acid, 4

To a solution of **15** (0.020 g, 0.042 mmol) in dry $CHCl_3$ (0.5 mL), trifluoroacetic acid (0.5 mL) and triethylsilane (0.02 mL, 0.13 mmol) were added at room temperature. The mixture was stirred for 3 days. Any excess trifluoroacetic acid was eliminated by adding CH_2Cl_2 and concentrating the mixture several times in vacuo. The residue was flash-chromatographed (0–10% methanol in chloroform) to afford **4** (0.004 g, 21%). ^{1}H NMR ($C_2D_2Cl_4$, 110 °C, 400 MHz): δ 0.86 (3H, d, $J = 6.7$ Hz, –CH$_3$), 1.16 (1H, m, H-4), 1.56 (1H, m, H′-4), 2.74 (1H, s, –NCH$_3$), 3.37 (2H, m, H-5 and H′-5), 4.10 (1H, t-like, $J = 6.7$ Hz, CH-Fmoc), 4.16 (1H, m, H-2), 4.27 (2H, br s, –C*H*$_2$Ph), 4.39 (2H, d-like, $J = 6.7$ Hz, CH_2-Fmoc), 7.16 (7H, m, Ar-Fmoc and Ar–Bn overlapped), 7.24 (2H, t-like, $J = 7.3$ Hz, Ar-Fmoc), 7.43 (2H, d-like, $J = 6.8$ Hz, Ar-Fmoc), 7.60 (2H, d-like, $J = 6.8$ Hz, Ar-Fmoc). ^{13}C NMR ($C_2D_2Cl_4$, 80 °C, 100 MHz): δ 14.7, 27.8, 27.9, 30.9, 45.7, 66.0, 66.1, 71.1, 71.9, 118.2 (×2), 123.1 (×2), 125.4 (×2), 125.7 (×3), 126.0 (×2), 126.5 (×2), 136.8, 139.5 (×2), 142.1 (×2), 155.2, 169.0. HR-ESMS m/z 474.2271 (calcd 474.2280 for $C_{29}H_{32}NO_5$). $[\alpha]_D^{25} = -52.2$ (c 0.2, $CHCl_3$).

Acknowledgements

This work has been supported by the MIUR ('Sostanze naturali ed analoghi sintetici con attività antitumorale') and by the Università degli Studi di Salerno.

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13. It is interesting to note that complete spectral elucidation (^{1}H and ^{13}C NMR) for compounds containing the 5-oxazolidinone ring **13**–**15** was possible at 80 °C or higher temperatures (see Experimental section).
14. No substantial improvement of the yields were noted when different reaction conditions were used: 9-BBN as hydroborating agent, excess of reagents (4.0–6.0 equiv), different reaction times (3–12 h) and temperatures of reaction (−10 °C to rt).
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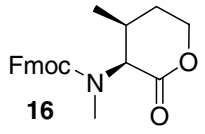
17. To overcome the problem of the low yielding benzyl protection and reductive oxazolidinone opening, we subjected compound **14** to a direct reductive oxazolidinone ring opening. Unfortunately, from the complex crude reaction mixture, only compound **16** ((3*S*,4*S*)-3-[(9H-fluoren-9-ylmethoxycarbonyl)-methylamino]-4-methyl-tetrahydro-2H-pyran-2-one) could be isolated in 30% yield. ^{1}H NMR ($C_2D_2Cl_4$, 110 °C, 400 MHz): δ 0.81 (3H, br, –CH_3), 1.16 (1H, br, H-5), 1.57 (1H, br, H′-5), 2.16 (1H, br, H-4), 2.87 (1H, br, –NCH_3), 3.37 (2H, m, H-5 and H′-5), 4.10–4.62 (5H, br, H-6, H′-6, CH-Fmoc, CH_2-Fmoc, H-3), 7.16 (7H, br, Ar-Fmoc), 7.24 (2H, br, Ar-Fmoc), 7.42 (2H, br, Ar-Fmoc), 7.59 (2H, br, Ar-Fmoc). ^{13}C NMR ($C_2D_2Cl_4$, 80 °C, 100 MHz): δ 14.0, 30.0, 33.2, 45.7, 65.0, 65.6, 72.1, 118.0 (×2), 123.0 (×2), 125.3 (×2), 125.7 (×3), 126.0 (×2), 126.5 (×2), 125.9, 139.5 (×2), 142.1 (×2), 154.2, 168.4. HR-ESMS m/z 366.1699 (calcd 366.1705 for $C_{22}H_{24}NO_4$). $[\alpha]_D^{25} = -75.3$ (c 0.5, $CHCl_3$)



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