Optically pure *N*-hydroxy-*O*-triisopropylsilyl- α -L-amino acid methyl esters from AlCl₃-assisted ring opening of chiral oxaziridines by nitrogen containing nucleophiles.

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General experimental methods

All reactions were carried out under an argon atmosphere. Glassware was cleaned overnight in a KOH/EtOH base bath, rinsed with MeOH and flame dried under an inert atmosphere before use in all anhydrous systems. Solvents were purified and dried by standard procedures,¹ stored on molecular sieves and distilled prior to use. Reagents were used without further purification. An exception was for *m*-chloroperoxybenzoic acid (*m*-CPBA), which was obtained dried by applying the procedure reported below. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ¹H and ¹³C NMR spectra were recorded at 300 and 75 MHz respectively, on dilute solution of each one sample dissolved in CDCl₃ or [d₆]DMSO as solvents. Chemical shifts (δ) are reported in ppm and referred to the tetramethylsilane resonance. Coupling constants (*J*) are reported in Hertz. FT-IR spectra were recorded on neat compounds or on KBr wafers. GC-MS analyses were performed using an HP-5MS (30 m $\times$ 0.25 mm, PhMesiloxane 5 %) capillary column. The mass detector operated in the electron impact ionization mode (EIMS) with an electron energy of 70 eV. Mass spectra were recorded by fast atom bombardment (FAB⁺ MS), using 3-nitrobenzyl alcohol (NBA) as matrix, with a neutral Xenon beam operating at 8 keV and a total current of 10 μ A. Exact mass determinations in high resolution mass spectrometry (HRMALDI) were acquired on a mass spectrometer equipped with a 200 Hz, Nd:YAG laser at 355 nm wavelength. Spectra were acquired in reflectron mode (20 keV accelerating voltage), with a 400 ns delayed extraction, averaging 2000 laser shots. CHCA (α -cyano-4-hydroxycinnamic acid) was used as matrix. Samples for HRMALDI were prepared by mixing 0.45 μ L of CHCA and the analyte dissolved in a 1:1 (v/v) MeOH/water solution, in a 800:1 matrix/analyte relative ratio. Samples were then spotted on the target, dried at room temperature and inserted into the mass spectrometer.

All reactions were monitored by thin layer chromatography (TLC) using silica gel 60-F₂₅₄ precoated

glass plates, without fluorescence indicator, and neutral alumina precoated aluminium plates. An UV lamp operating at 254 nm, and/or spraying with a 20 % (v/v) solution of H₂SO₄ in absolute ethanol and charring at 180 °C for 1 min. was used to visualize spots on TLC plates. Oximes and oximates were visualized as previously reported by Møller.² A 1 % (w/w) solution of alizarine in absolute ethanol was used to visualize the free hydroxylamines as yellow-orange spots. The Folin-Ciocalteu phenol reagent³ was used to unequivocally visualize *N*-hydroxy- α -L-amino acid methyl ester and their *O*-triisopropyl derivatives as intense blue coloured spots, respectively. Ninhydrin was used to visualize amino acid methyl esters as deep pink coloured spots. Kieselgel 60H without gypsum, and neutral alumina when required, were used as stationary phases for flash column chromatography (FCC). Purification were performed applying the same gradients optimized for TLC, but reducing to ¼ the polar component of each one eluent mixture.

Optical rotations of all new chiral compounds (oxaziridines and *N*-hydroxy-*O*-triisopropylsilyl- α -L-amino acid methyl esters) were measured in a 1 dm (2 mL volume) tube. All measurements were carried out on chloroform solutions of each compound (*c* 2), and $[\alpha]_D$ values were determined at 20 °C. The $[\alpha]_D$ values obtained for oxaziridines matched those predictable by the Freudenberg's rule⁴ for the same compounds.

Preparation of anhydrous *m*-chloroperoxybenzoic acid.

Commercial available *m*-CPBA was dissolved in methylene chloride (CAUTION!), and the resulting heterogeneous suspension was filtered under vacuum to remove the insoluble *m*-chlorobenzoic acid. The organic solution was washed three times with brine, dried on MgSO₄ and the solvent evaporated to dryness under reduced pressure conditions. The white solid recovered was recrystallized from a 1:2 (v/v) methyl-*tert*-butyl ether/*n*-pentane solution. After filtration, crystals were collected, washed once again with cold *n*-pentane and stored on P₂O₅ under vacuum overnight.

Preparation of free *O*-benzyl hydroxylamine.

The commercially available *O*-benzyl hydroxylamine hydrochloride (1 equiv) was treated (CAUTION!) with MeONa (1 equiv) in MeOH (10 mL), under magnetic stirring for 10 min at 0 °C. After removal of the solvent under reduced pressure conditions, the white powder obtained was suspended in MeOH (5 mL) and filtered through a glass column filled with CELITE[®] 545, using MeOH as eluent. The mother liquor was evaporated to dryness under reduced pressure conditions to give the required nucleophile as a white sticky solid.

Synthesis of oxaziridines 18-20. General procedure.

The appropriate imine (5.75 mmol) was dissolved in methylene chloride (15 mL) and maintained at 0 °C under magnetic stirring. A solution of anhydrous *m*-chloroperoxybenzoic acid (1.1 g, 6.33 mmol) in methylene chloride (15 mL) was added dropwise in 20 min. After stirring for 23 h at -40 °C, TLC showed the reaction to be complete (Et₂O/*n*-hexane 60:40, v/v), and *m*-chlorobenzoic acid precipitated was rapidly removed by filtration under vacuum. The mother liquor was treated with a 1 N NaOH aqueous solution (20 mL) and brine (10 mL), and then extracted with ethanol free chloroform (5 × 10 mL). Organic layers were separated, washed once again with brine (10 mL), and dried on MgSO₄. Solvent was removed under reduced pressure condition and the mixture of diastereomeric oxaziridines so recovered was subjected to spectral characterization. ¹H NMR spectra were characterized by an extended overlapping of the resonances for the stereomers obtainable in each case, and only the signals attributable to the methyl group of the ester handle in the oxaziridine frames were well distinguished in all cases. Relative percentage product distributions and *trans/cis* product ratios for each mixture of oxaziridines were determined on the basis of the intensity of resonances attributable to the methyl group in the ester moiety of each oxaziridine, and assignments were confirmed in analogy to oxaziridines **2a-5a** and **2b-5b**.

All imine oxidations produced a pale yellow oily product which was pure enough to be employed in the next step. Overall yields: 70-77 %.

Oxaziridines 18. (1.174 g, 4.43 mmol; 77 %). Mixture of diastereomers; rel. % product distribution: 63:23:9:5; *trans/cis* product ratio: 86:14. TLC (Et₂O/*n*-hexane 60:40, v/v) *R_f* = 0.58, 0.56, 0.55, 0.53. FT-IR 1752 (broad) cm⁻¹. ¹H NMR (CDCl₃) δ : 7.28-7.36 (m, 8 H), 6.70-6.78 (m, 8 H), 4.65 (s, 2 H), 4.62 (s, 2 H), 3.79-3.81 (m, 12 H), 3.76 (s, 3 H), 3.75 (s, 3 H), 3.72 (s, 3 H), 3.70 (s, 3 H), 3.06-3.44 (m, 4 H), 2.65-2.75 (m, 4 H), 0.97-1.11 (m, 24 H). ¹³C NMR (CDCl₃) δ 172.8, 172.4, 172.3, 172.0, 159.5, 159.3, 129.2, 129.1, 129.0, 127.8, 127.5, 114.7, 114.3, 114.1, 80.3, 80.1, 79.4, 79.1, 73.2, 73.1, 72.8, 72.5, 56.4, 56.3, 52.7, 52.5, 24.7, 24.5, 18.1, 18.0. MS (EI) *m/z* (rel. intensity %) 265 (M⁺, 5.4), 250 (12.1), 234 (6.7), 222 (8.9), 206 (22.2), 158 (23.4), 150 (34.7), 115 (18.9), 107 (87.9), 76 (23.4), 59 (31.0), 43 (100), 31 (19.8). HRMALDI: Calcd. for C₁₄H₂₀NO₄ (M+H)⁺, 266.13923; found 266.13919.

Oxaziridines 19. (1.021 g, 4.31 mmol, 75 %). Mixture of diastereomers; rel. % product distribution: 57:26:10:7; *trans/cis* product ratio: 83:17. TLC (Et₂O/*n*-hexane 60:40, v/v) *R_f* = 0.53, 0.52, 0.50, 0.49. FT-IR 1750 (broad) cm⁻¹. ¹H NMR (CDCl₃) δ 7.27-7.35 (m, 8 H), 6.71-6.78 (m, 8 H), 4.64 (s, 2 H), 4.62 (s, 1 H), 4.61 (s, 1 H), 3.79-3.82 (m, 12 H), 3.75 (s, 3 H), 3.74 (s, 3 H), 3.71 (s, 3 H), 3.69 (s, 3 H), 3.08-3.46 (m, 4 H), 1.23-1.29 (m, 12 H). ¹³C NMR (CDCl₃) δ 172.7, 172.4, 172.3, 172.0, 159.5, 159.3, 129.1, 129.0, 127.8, 127.5, 114.9, 114.7, 80.9, 79.4, 78.4, 79.0, 63.8, 63.5, 63.0, 62.8, 56.8, 56.2, 56.1, 52.7, 52.5, 52.0, 12.0, 11.8. MS (EI) *m/z* (rel. intensity %) 237 (M⁺, 8.9), 222 (10.9), 206 (23.4), 178 (5.8), 150 (8.9), 130 (12.1), 107 (100), 87 (23.4), 76 (45.0), 59 (22.0), 31 (15.6). HRMALDI: Calcd. for C₁₂H₁₆NO₄ (M+H)⁺, 238.10793; found 238.10789.

Oxaziridines 20. (0.899 g, 4.03 mmol, 70 %). Mixture of diastereomers; rel. % product distribution: 55:45; *trans/cis* product ratio: 55:45. TLC (Et₂O/*n*-hexane 60:40, v/v) *R_f* = 0.46, 0.45, 0.44, 0.43. FT-IR 1750 (broad) cm⁻¹. ¹H NMR (CDCl₃) δ 7.28-7.36 (m), 6.68-6.90 (m), 4.65 (s), 4.62 (s), 3.80 (s), 3.78 (s), 3.72 (s), 3.69 (s), 3.49-3.52 (m, 4 H, α-CH₂). ¹³C NMR (CDCl₃) δ 170.6, 170.4, 159.5, 129.5, 129.3, 127.4, 127.0, 114.4, 114.2, 78.4, 78.2, 60.1, 59.8, 56.4, 56.3, 52.0, 51.8. MS (EI) *m/z* (rel. intensity %) 223 (M⁺, 5.0), 208 (7.5), 192 (8.8), 164 (12.1), 150 (23.4), 116 (19.0), 107 (100), 76 (22.6), 73 (17.8), 59 (28.9), 31 (12.3). HRMALDI: Calcd. for C₁₁H₁₄NO₄ (M+H)⁺, 224.09228; found 224.09221.

Characterization of *O*-benzyl aldoximates 14 and 15.

14. Pale yellow oil, TLC (Et₂O/*n*-hexane 60:40, v/v) *R_f* = 0.81. FT-IR 3061, 3029, 2963, 2870, 1711, 1679, 1606, 1571, 1454, 1306, 1251, 1170, 1032, 937, 830, 786 cm⁻¹. ¹H NMR (CDCl₃) δ 8.13 (s, 1 H), 7.54 (d, 2 H, *J* = 8.0), 7.11-7.38 (m, 5 H), 6.95 (d, 2 H, *J* = 8.0), 5.23 (s, 2 H), 3.80 (s, 3 H). ¹³C NMR (CDCl₃) δ 163.2, 153.9, 141.2, 130.6, 129.0, 127.7, 127.2, 126.0, 114.5, 75.2, 55.9. MS (EI) *m/z* (rel. intensity %) 241 (M⁺, 62.6), 211 (12.7), 198 (10.4), 134 (3.6), 105 (27.0), 91 (100), 77 (39.5), 65 (4.9), 51 (3.6). HRMALDI: Calcd. for C₁₅H₁₆NO₂ (M+H)⁺, 242.11810; found 242.11800.

15. Pale yellow oil, TLC (Et₂O/*n*-hexane 60:40, v/v) *R_f* = 0.79. FT-IR 3029, 2963, 2930, 2836, 1710, 1679, 1571, 1513, 1454, 1251, 1170, 1032, 937, 914, 830, 786 cm⁻¹. ¹H NMR (CDCl₃) δ 7.98 (s, 1 H), 7.56 (d, 2 H, *J* = 8.0), 7.12-7.40 (m, 5 H), 6.97 (d, 2 H, *J* = 8.0), 5.28 (s, 2 H), 3.81 (s, 3 H). ¹³C NMR (CDCl₃) δ 163.2, 149.9, 140.2, 130.5, 128.7, 127.7, 127.2, 126.0, 112.3, 75.4, 56.1. MS (EI) *m/z* (rel. intensity %) 241 (M⁺, 58.9), 211 (12.5), 198 (11.2), 134 (4.6), 105 (27.8), 91 (100), 77 (41.2), 65 (5.0), 51 (4.2). HRMALDI: Calcd. for C₁₅H₁₆NO₂ (M+H)⁺, 242.11810; found 242.11800.

Synthesis of *N*-hydroxy-*O*-triisopropylsilyl- α -L-amino acid methyl esters 23-25. General procedure.

To a solution of the appropriate oxaziridine (as mixture of diastereoisomers; 2.2 mmol) dissolved in methylene chloride (10 mL), AlCl_3 (0.59 g, 4.4 mmol) was added. After 5 min. standing at r.t., a solution of free *O*-benzyl hydroxylamine (0.6 g; 2.2 mmol) in methylene chloride (10 mL) was added and the resulting mixture was refluxed under magnetic stirring for 25 min. After this time, TLC showed reaction to be complete ($\text{Et}_2\text{O}/n$ -hexane 60:40, v/v), and the solution was cooled at r.t., then treated with a 1 N HCl aqueous solution (pH = 3). The aqueous phase was separated and the organic layer, after drying on MgSO_4 , was filtered and directly treated with a methylene chloride (10 mL) solution containing 1*H*-imidazole (0.15 g, 2.2 mmol) and triisopropylsilyl triflate (TIPSOTf; 0.52 mL, 2.2 mmol), under magnetic stirring at r.t. Reaction was complete in 3 h, as checked by TLC ($\text{Et}_2\text{O}/n$ -hexane 60:40, v/v). The organic solvent was removed under reduced pressure conditions and the solid residue was dissolved in a 5 % NaHCO_3 aqueous solution (5 mL). The aqueous phase was extracted with AcOEt (3×5 mL), and the collected organic layers were washed once with a 5 % KHSO_4 aqueous solution (5 mL) and once with brine (5 mL). After drying on MgSO_4 , the solvent was removed under reduced pressure conditions to give a pale yellow crude material, which was chromatographed to afford the title compounds in 88-94% overall yields.

***N*-hydroxy-*O*-triisopropylsilyl- α -L-amino acid methyl ester 23.** White powder (0.63 g, 2.07 mmol; 94 %), mp = 107-109 °C. $[\alpha]_D^{20} = +15.8$. TLC ($\text{Et}_2\text{O}/n$ -hexane 60:40, v/v) $R_f = 0.68$. FT-IR (KBr) 3120, 2965, 2774, 1758, 1209 cm^{-1} . ^1H NMR (CDCl_3) δ 6.07 (d, 1 H, $J = 10.5$), 3.67 (s, 3 H), 3.18 (dd, 1 H, $J = 10.5$, $J = 7.6$), 1.65-1.85 (m, 4 H), 0.81-0.87 (m, 24 H). ^{13}C NMR (CDCl_3) δ 173.6, 72.4, 52.1, 27.0, 18.2, 17.1, 10.5. MS (EI) m/z (rel. intensity %) 303 (M^+ , 18.8), 288 (12.8), 272 (11.0), 260 (8.9), 244 (3.5), 188 (45.8), 173 (12.4), 157 (21.1), 146 (23.1), 130 (65.4), 115 (21.4), 59 (24.3), 43 (100), 31 (4.5).

HRMALDI: Calcd. for $C_{15}H_{34}NO_3Si$ ($M+H$)⁺, 304.23080; found 304.23073.

***N*-hydroxy-*O*-triisopropylsilyl- α -L-amino acid methyl ester 24.** White powder (0.551 g, 2.0 mmol; 91 %), mp = 115-117 °C. $[\alpha]_D^{20} = +16.2$. TLC (Et₂O/*n*-hexane 60:40, v/v) $R_f = 0.67$. FT-IR (KBr) 3109, 2963, 2771, 1756, 1212 cm⁻¹. ¹H NMR (CDCl₃) δ 6.18 (d, 1 H, $J = 10.5$), 3.71-3.79 (m, 1 H), 3.67 (s, 3 H), 1.82 (septet, 3 H, $J = 7.2$), 1.28 (d, 3 H, $J = 7.1$), 0.88 (d, 18 H, $J = 7.2$). ¹³C NMR (CDCl₃) δ 173.4, 62.3, 52.7, 17.7, 13.8, 10.4. MS (EI) m/z (rel. intensity %) 275 (M^+ , 32.0), 260 (21.5), 244 (22.3), 232 (12.9), 216 (11.7), 188 (23.6), 173 (65.9), 157 (3.5), 118 (5.8), 102 (7.9), 87 (23.7), 59 (34.8), 43 (100), 31 (2.3). HRMALDI: Calcd. for $C_{13}H_{30}NO_3Si$ ($M+H$)⁺, 276.19950; found 276.19944.

***N*-hydroxy-*O*-triisopropylsilyl- α -L-amino acid methyl ester 25.** Colorless oil (0.506 g, 1.94 mmol; 88 %), TLC (Et₂O/*n*-hexane 60:40, v/v) $R_f = 0.59$. FT-IR (KBr) 3110, 2938, 2758, 1757, 1209 cm⁻¹. ¹H NMR (CDCl₃) δ 6.18 (d, 1 H, $J = 10.2$), 4.22 (d, 2 H, $J = 10.2$), 3.71 (s, 3 H), 1.81 (septet, 3 H, $J = 7.1$), 0.89 (d, 18 H, $J = 7.1$). ¹³C NMR (CDCl₃) δ 172.9, 57.3, 52.4, 17.6, 10.2. MS (EI) m/z (rel. intensity %) 261 (M^+ , 15.6), 246 (12.1), 230 (3.5), 218 (8.9), 202 (15.8), 173 (43.8), 157 (23.6), 104 (18.0), 88 (11.1), 59 (56.8), 43 (100), 31 (32.3). HRMALDI: Calcd. for $C_{12}H_{28}NO_3Si$ ($M+H$)⁺, 262.18385; found 262.28380.

References cited in the general experimental methods.

- [1] Furniss, B.S.; Hannaford, A.G.; Smith, P.W.G.; Thatchell, A.R. *Vogel's Textbook of Practical Organic Chemistry*, 5th Edn; Longman Scientific and Technical; J. Wiley & Sons, New York; 1989.
- [2] Møller, B.L. *Anal. Biochem.* **1977**, *81*, 292.
- [3] Ikawa, M.; Schaper, T.D.; Dollard, C.A.; Sasner, J.J. *J. Agric. Food Chem.* **2003**, *51*, 1811.
- [4] Freudenberg, K. *Chem. Ber.* **1933**, *66*, 177.