6 Experimental

6.1 General Experimental Details

Reagents were used as supplied or purified using standard procedures as necessary. Solvents used in solid-phase peptide synthesis (SPPS) and during the tandem cyclisation step were purchased as reagent and anhydrous grades, respectively, and used without further purification. Acetonitrile (MeCN) used in analytical liquid chromatography-mass spectrometry (LCMS) and preparative reversed-phase high-performance liquid chromatography (RP-HPLC) was purchased as HPLC grade from Merck Millipore and RCI Labscan, respectively. Water used throughout was ultrapure (Type 1), purified by a Milli-Q® purification system.

Melting points were determined using a Thermo Scientific 9200 (IA9200X6) digital melting point apparatus. Optical rotations were measured on a JASCO DIP-1020 digital polarimeter using a sodium lamp (589 nm) as the light source, the [α] values reported are averaged from 10 repeats and reported in 10^{-1}·deg·cm^{2}·g^{-1}. Infrared spectra were recorded as thin films on a Perkin Elmer Spectrum One FT-IR spectrometer fitted with a Universal ATR sampling accessory. ^{1}H and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively, on a Bruker AVANCE 600 spectrometer. *J* values are given in Hz. ^{13}C DEPT-90 and 135 and two-dimensional (COSY, ed-HSQC and HMBC) NMR experiments were used, where appropriate, to support the assignment of signals in the ^{1}H and ^{13}C spectra.

Analytical LCMS: High-performance liquid chromatography-mass spectrometry (HPLC-MS) was conducted on a Bruker amaZon SL ion trap mass spectrometer with an ESI source, equipped with a Dionex UltiMate 3000 HPLC system using a reversed-phase Dionex Acclaim® 120 C18 column (3 µm, 150 × 2.1 mm) at a flow rate of 0.2 ml/min (gradient: 0-100% MeCN with 0.1% formic acid in H_2O with 0.1% formic acid, 40 min). A programmed method involving 15-min column equilibration with the initial condition of 100% aqueous (H_2O with 0.1% formic acid), followed by a gradient profile of 100% aqueous held for 1 min then linearly increased to 100% organic (MeCN with 0.1% formic acid) in 40 min, and finally washing the column with 100% organic for 10 min, was employed. Each sample was prepared from diluting a filtered reaction mixture (approx. 1 ml) with MeCN to a resulting volume of 5 ml (volumetric flask), 100 µl of which was further diluted with MeCN to reach a total volume of 1000 µl. Additional 5 µl of a solution of a known cyclic peptide (1 mg/ml) was then added to the 50-fold diluted sample as a reference compound. The sample injection volume was 5 µl. The ESI–MS data were acquired in the positive ionisation mode. The capillary voltage was set to 3500 V. Nitrogen (N_2) was used as both drying gas (flow rate of 10 l/min, 160 °C) and nebulising gas (30 psi) at a full scan range of *m/z*

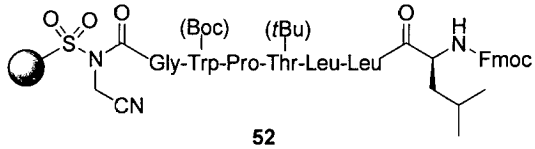
100-2000. Each sample was analysed at least twice to obtain an average number of counts of the sum of the $[M+H]^+$ and $[M+Na]^+$ ions of **53** at *m/z* 937.6 and 959.6, respectively.

Preparative RP-HPLC: Preparative reversed-phase high-performance liquid chromatography was conducted on a Thermo Separation Products HPLC system, including a SpectraSYSTEM P4000 pump and a SpectraSYSTEM UV2000 detector, using a reversed-phase Grace Vydac® 218TP™ C18 column (10 µm, 250 × 22 mm) with a compatible guard cartridge at a flow rate of 11 ml/min (gradient: 0-100% MeCN in H_2O, 40 min, 10-70% MeCN in H_2O, 40 min or 50-70% MeCN in H_2O, 40 min) with detection at 230 and 280 nm. For each RP-HPLC cycle, the column was equilibrated with an initial condition for 10-20 min before starting a gradient profile (injection of a sample) and finally flushed with 100% MeCN for 10-20 min.

HRMS was performed using time-of-flight mass spectrometry (TOF-MS) on a Bruker microTOF mass spectrometer in the positive electrospray ionisation (ESI) mode. The capillary voltage was set to 3500 V. Nitrogen (N_2) was used as both drying gas (flow rate of 8 l/min, 120 °C) and nebulising gas (2 bar). Sodium formate was used as the internal mass calibrant. The instrument was set to a resolution of 9000-10000 for the entire mass range. Samples were introduced via a microsyringe pump at a flow rate of 480 µl/h.

6.2 Experimental Procedures

Solid-supported activated peptide (**52**)



52

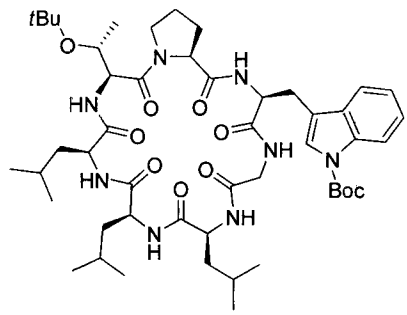
Solid-phase peptide synthesis: To 4-sulfamylbutyryl AM resin (**5**, Novabiochem, 0.90 mmol/g, 0.55 g, 0.5 mmol) pre-swollen in DMF was added a pre-mixed solution of Fmoc-Gly-OH (0.59 g, 2.0 mmol, 4 eq), HBTU (0.76 g, 2.0 mmol, 4 eq) and DIPEA (700 µl, 4.0 mmol, 8 eq) in DMF (4.5 ml, 0.45 M). The mixture was agitated for 3 h. The resin was drained under suction and washed with DMF (3×). The first coupling was repeated once more where the reaction mixture was left overnight. A pre-mixed solution of capping reagents, HOBt (0.27 g, 2.0 mmol, 4 eq), acetic anhydride (Ac_2O; 190 µl, 2.0 mmol, 4 eq) and DMAP (cat) in DMF (4.5 ml), was added. The reaction mixture was agitated for 2 h before the resin was drained under suction, washed with DMF (3×) and DCM (3×), and left to dry. The dried resin (1.0 mg) was subjected to Fmoc removal UV analysis to determine the loading efficiency. The rest of the resin (**49**) was swollen in

DMF again. The Fmoc group was removed by treating the resin (**49**) with 20% piperidine in DMF for 30 min. The resin was drained under suction and washed with DMF (5×) to achieve **50**. The presence of the amino group was qualitatively detected by the ninhydrin test to ensure Fmoc removal. A pre-mixed solution of Fmoc-Trp(Boc)-OH (1.05 g, 2.0 mmol, 4 eq), HBTU (0.76 g, 2.0 mmol, 4 eq) and DIPEA (700 µl, 4.0 mmol, 8 eq) in DMF (4.5 ml) was added and the resulting reaction mixture agitated for 3 h. After draining under suction and washing with DMF (3×), the resin was ninhydrin tested before subjecting to Fmoc removal using 20% piperidine in DMF for 30 min. The resin was drained under suction, washed with DMF (5×) and ninhydrin tested before the coupling and deprotection cycles were repeated for Fmoc-Pro-OH (0.67 g, 2.0 mmol, 4 eq), Fmoc-Thr(*t*-Bu)-OH (0.79 g, 2.0 mmol, 4 eq), Fmoc-Leu-OH (0.71 g, 2.0 mmol, 4 eq), and Fmoc-Leu-OH (0.71 g, 2.0 mmol, 4 eq). The third Fmoc-Leu-OH unit was then coupled in the same manner to obtain the requisite linear peptide **51**, which was then washed with *N*-methyl-2-pyrrolidone (NMP, 5×).

NB: In the ninhydrin test (the Kaiser test),[88, 89] the presence of the amino group after Fmoc removal yields blue and the absence which suggests a successful amino acid coupling gives no colour change to the resin.

Safety-catch linker activation: To the resulting linear peptide resin **51**, pre-swollen in NMP, was added DIPEA (870 µl, 5.0 mmol, 10 eq), followed by iodoacetonitrile (ICH_2CN; 730 µl, 10.0 mmol, 20 eq) freshly filtered through a plug of basic alumina. The reaction mixture was agitated for 30 min and left to stand in the dark (wrapped with aluminum foil) at rt overnight. The resin was drained, washed with DMF (5×) and DCM (3×) and dried, first under suction for 1 h and then *in vacuo*, to achieve the activated resin **52** (1.05 g *cf.* the theoretical mass of 1.1548 g calculated from the manufacturer's stated resin substitution of 0.90 mmol/g for 0.5 mmol of the starting resin **5**).

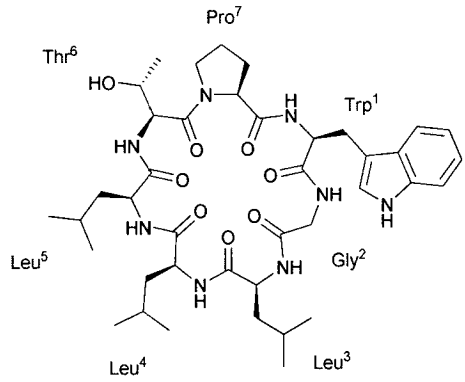
Protected cyclic peptide intermediate (53)



This reaction was performed under an argon atmosphere using oven-dried glassware, which was further dried in vacuo with a heat gun, once charged with 4-Å molecular sieves, to achieve anhydrous conditions.

To a round-bottomed flask charged with activated 4-Å molecular sieves was added activated resin **7** (0.56 g, 0.24 theoretical mmol), followed by anhydrous DMF (100 ml). The resin was allowed to swell for 30 min prior to addition of TEA (distilled over CaH_2 and kept over KOH; 0.4 ml, 10 eq). The reaction mixture was stirred at rt for 7 d and then filtered through a plug of Celite, washed with MeCN (50 ml) and DCM (70 ml). The filtrate was concentrated under reduced pressure to remove volatile solvents (diaphragm pump) and as much DMF as possible (high-vacuum oil pump). The resulting DMF solution was subjected to preparative RP-HLPC to isolate cyclic peptide **8** (0-100% MeCN in H_2O, 40 min, t_R 36 min; then 50-70% MeCN in H_2O, 40 min, t_R 27 and 31 min) as a white solid (46 mg, 21% yield). mp 192 °C; $[\alpha]_D^{24}$ –69.6 (*c* 1.015 in MeOH); UV (MeOH) λ_{max} (log ϵ) 293 (0.18), 286 (0.17), 263 (0.29), 259 (0.29), 228 (0.77), 202 (1.19); IR (film) ν_{max}/cm^{-1} 3286, 3058, 2958, 2932, 2871, 1735, 1637, 1528, 1453, 1369, 1330, 1309, 1256, 1228, 1191, 1158, 1125, 1085, 1044, 1017, 858, 805, 768, 746, 674; ^{1}H NMR (600 MHz, DMSO-d_6) δ_H 0.80-0.93 (18H, m, 6×Leu-CδH_3), 1.03 (3H, d, *J* 6.6, Thr-CγH_3), 1.07 (9H, s, Thr-OC(CH_3)$_3$), 1.38-1.60 (6H, m, 3×Leu-CβH_2), 1.38-1.52 (2H, m, Pro-CγH_2), 1.45-1.60 (2H, m, Leu^3-CγH and Leu^5-CγH), 1.61 (9H, s, Trp-CO_2C(CH_3)$_3$), 1.66-1.73 (2H, m, Pro-CβHH' and Leu^4-CγH), 1.84 (1H, br dd, *J* 12.0, 6.6, Pro-CβH*H'*), 2.88-2.93 (1H, m, Pro-CδHH'), 3.14-3.20 (2H, m, Pro-CδH*H'* and Trp-CβHH'), 3.28-3.32 (1H, m, Trp-CβH*H'*), 3.85-3.92 (2H, m, Leu^3-CαH and Thr-CβH), 3.95 (2H, br d, Gly-CαH_2), 4.23 (1H, app td, *J* 9.8, 4.5, Leu^4-CαH), 4.32 (1H, br dd, *J* 5.4, 2.4, Thr-CαH), 4.45 (1H, ddd, *J* 12.0, 7.8, 3.6, Trp-CαH), 4.49 (1H, dd, *J* 14.4, 7.2, Leu^5-CαH), 5.02 (1H, d, *J* 7.8, Pro-CαH), 7.04 (1H, br d, *J* 7.2, Leu^5-N*H*), 7.23 (1H, t, *J* 7.8, Trp-C$_{5'}H$), 7.33 (1H, t, *J* 7.8, Trp-C$_{6'}H$), 7.39 (1H, s, Trp-C$_{2'}H$), 7.58 (1H, d, *J* 7.8, Trp-C$_{4'}H$), 7.63 (1H, br t, Gly-N*H*), 7.93 (1H, br d, *J* 9.8, Leu^4-N*H*), 8.06 (1H, d, *J* 7.8, Trp-C$_{7'}H$), 8.12 (1H, d, *J* 7.8, Trp-N*H*), 8.29 (1H, br d, *J* 4.2, Leu^3-N*H*), 8.45 (1H, br d, *J* 5.4, Thr-N*H*); ^{13}C NMR (150 MHz, DMSO-d_6) δ_C 19.4 (Thr-*C*γH_3), 20.9 (Pro-*C*γH_2), 20.9, 21.2, 22.4, 22.6, 22.8 and 22.9 (6×Leu-*C*δH_3), 24.1, 24.2 and 24.5 (3×Leu-*C*γH), 26.4 (Trp-*C*βH_2), 27.5 and 27.6 (Thr-OC(*C*H_3)$_3$ and Trp-CO_2C(*C*H_3)$_3$), 30.4 (Pro-*C*βH_2), 39.8, 39.8 and 40.5 (3×Leu-*C*βH_2), 42.0 (Gly-*C*αH_2), 45.9 (Pro-*C*δH_2), 50.6 (Leu^5-*C*αH), 51.7 (Leu^4-*C*αH), 53.5 (Leu^3-*C*αH), 54.3 (Trp-*C*αH), 57.2 (Thr-*C*αH), 60.0 (Pro-*C*αH), 67.2 (Thr-*C*βH), 74.3 (Thr-O*C*(CH_3)$_3$), 83.6 (Trp-CO_2*C*(CH_3)$_3$), 114.6 (Trp-*C*$_{7'}$H), 116.8 (Trp-*C*$_{3'}$), 118.8 (Trp-*C*$_{4'}$H), 122.7 (Trp-*C*$_{5'}$H), 123.5 (Trp-*C*$_{2'}$H), 124.4 (Trp-*C*$_{6'}$H), 129.7 (Trp-*C*$_{3a'}$), 134.8 (Trp-*C*$_{7a'}$), 148.8 (Trp-*C*O_2C(CH_3)$_3$), 167.9 (Pro-*C*O), 169.1 (Leu^3-*C*O), 170.6 (Gly-*C*O), 171.0 (Trp-*C*O), 171.6 (Leu^5-*C*O), 171.7 (Thr-*C*O), 172.0 (Leu^4-*C*O); HRMS (ESI-TOF) *m/z* $[M+Na]^+$ Calcd for $C_{49}H_{76}N_8O_{10}Na^+$ 959.5577; found 959.5573.

Integerrimide A (40)



To **53** (24 mg, 0.025 mmol) was added a pre-mixed solution of 95:2.5:2.5 TFA/TIPS/H_2O (25 ml) under Ar. The resulting solution was stirred at rt for 1 h before diluting with DCM (30 ml). After removing all volatiles under reduced pressure, the crude residue was subjected to preparative RP-HPLC for purification (0-100% MeCN in H_2O, 40 min, t_R 23 and 28 min; and 50-70% MeCN in H_2O, 40 min, t_R 32 min) to afford the desired natural product (**1**) as a white solid upon concentration *in vacuo* (17 mg, 88% yield). mp 185.6-186.4 °C; $[\alpha]_D^{27}$ –75.6 (*c* 0.685 in MeOH) (lit.,[73] $[\alpha]_D^{25}$ –76.6, *c* 0.5 in MeOH); IR (film) ν_{max}/cm^{-1} 3301, 3058, 2957, 2932, 2871, 1638, 1526, 1434, 1368, 1340, 1263, 1249, 1186, 1151, 1120, 1012, 923, 741; ^{1}H NMR (600 MHz, DMSO-d_6) δ_H (major conformer) 0.40-0.49 (1H, m, Pro-C$_\gamma$*H*H'), 0.80 and 0.83 (3H, d, *J* 5.9 and 3H, d, *J* 6.6, Leu^3-C$_\delta$*H*$_3$ and Leu^4-C$_\delta$*H*$_3$), 0.84-0.87 (3H, ov, Leu^3-C$_{\delta'}$*H*$_3$), 0.89 and 0.90 (3H, d, *J* 6.2 and 3H, d, *J* 6.7, Leu^5-C$_\delta$*H*$_3$ and Leu^4-C$_{\delta'}$*H*$_3$), 0.95 (3H, d, *J* 6.3, Leu^5-C$_{\delta'}$*H*$_3$), 1.05 (3H, d, *J* 5.8, Thr-C$_\gamma$*H*$_3$), 1.29-1.35 (1H, m, Pro-C$_\gamma$H*H*'), 1.37-1.45 (1H, m, Leu^5-C$_\beta$*H*H'), 1.37-1.66 (4H, m, Leu^3-C$_\beta$*H*$_2$ and Leu^4-C$_\beta$*H*$_2$), 1.45-1.54 (1H, m, Leu^4-C$_\gamma$*H*), 1.55-1.66 (3H, m, Pro-C$_\beta$*H*H', Leu^5-C$_\beta$H*H*' and Leu^5-C$_\gamma$*H*), 1.66-1.72 (1H, m, Leu^3-C$_\gamma$*H*), 1.84 (1H, br dd, *J* 10.6, 7.7, Pro-C$_\beta$H*H*'), 2.41-2.48 (1H, m, Pro-C$_\delta$*H*H'), 3.04 (1H, app td, *J* 11.1, 7.6, Pro-C$_\delta$H*H*'), 3.15 (1H, dd, *J* 14.3, 12.0, Trp-C$_\beta$*H*H'), 3.30 (1H, dd, *J* 14.3, 3.5, Trp-C$_\beta$H*H*'), 3.83 (1H, dt, *J* 9.9, 4.9, Leu^3-C$_\alpha$*H*), 3.85 (1H, ov dd, *J* 5.2, 17.5, Gly-C$_\alpha$*H*H'), 3.88 (1H, quint, *J* 5.8, Thr-C$_\beta$*H*), 4.01 (1H, dd, *J* 17.5, 3.2, Gly-C$_\alpha$H*H*'), 4.22 (1H, ddd, *J* 12.8, 7.6, 3.8, Leu^4-C$_\alpha$*H*), 4.32 (1H, t, *J* 5.1, Thr-C$_\alpha$*H*), 4.35 (1H, ddd, *J* 12.0, 7.4, 3.5, Trp-C$_\alpha$*H*), 4.50 (1H, q, *J* 7.1, Leu^5-C$_\alpha$*H*), 4.76 (1H, d, *J* 7.7, Pro-C$_\alpha$*H*), 5.10 (1H, br d, *J* 5.8, Thr-O*H*), 6.95 (1H, br t, *J* 8.0, Trp-C$_{5'}$*H*), 6.98-7.00 (2H, br m, Trp-C$_{2'}$*H* and Leu^5-N*H*), 7.05-7.07 (1H, m, Trp-C$_{6'}$*H*), 7.32 (1H, d, *J* 8.0, Trp-C$_{7'}$*H*), 7.47 (1H, d, *J* 8.0, Trp-C$_{4'}$*H*), 7.63 (1H, br m, Gly-N*H*), 7.96-7.99 (2H, br m, Trp-N*H* and Leu^4-N*H*), 8.36 (1H, br d, *J* 4.9, Leu^3-N*H*), 8.59 (1H, d, *J* 5.1, Thr-N*H*), 10.93 (1H, br s, Trp-N$_{1'}$*H*); δ_H (minor conformer) 0.79-0.86 (12H, m, Leu^3-C$_\delta$*H*$_3$, Leu^3-C$_{\delta'}$*H*$_3$, Leu^4-C$_\delta$*H*$_3$ and Leu^4-C$_{\delta'}$*H*$_3$), 0.84-0.87 (6H, m, Leu^5-C$_\delta$*H*$_3$ and Leu^5-C$_{\delta'}$*H*$_3$), 1.10 (3H, d, *J* 6.3, Thr-C$_\gamma$*H*$_3$), 1.23 (1H, ov, Pro-C$_\beta$*H*H'), 1.37-1.66 (6H, ov m, Leu^3-C$_\beta$*H*$_2$, Leu^4-C$_\beta$*H*$_2$ and Leu^5-C$_\beta$*H*$_2$), 1.45-1.54 (3H, m, Leu^3-C$_\gamma$*H*, Leu^4-C$_\gamma$*H* and Leu^5-C$_\gamma$*H*), 1.45-1.72 (2H, ov m, Pro-C$_\gamma$*H*$_2$), 1.91-1.98 (1H, m, Pro-C$_\beta$H*H*'), 2.96-3.01 (1H, m, Trp-C$_\beta$*H*H'), 3.30 (1H, ov, Trp-C$_\beta$H*H*'), 3.34-3.48 (1H, m, Pro-C$_\delta$*H*H'), 3.58-3.72 (1H, m, Pro-C$_\delta$H*H*'), 3.69-3.73 (1H, m, Gly-C$_\alpha$*H*H'), 3.81-3.86 (1H,

ov m, Leu^4-C$_\alpha$*H*), 3.85-3.90 (1H, m, Gly-C$_\alpha$H*H'*), 4.04-4.07 (1H, ov, Leu^5-C$_\alpha$*H*), 4.09-4.16 (1H, m, Pro-C$_\alpha$*H*), 4.11-4.19 (1H, m, Thr-C$_\beta$*H*), 4.16-4.22 (1H, m, Leu^3-C$_\alpha$*H*), 4.30-4.34 (1H, ov, Thr-C$_\alpha$*H*), 4.30-4.37 (1H, ov, Trp-C$_\alpha$*H*), 6.97 (1H, ov, Trp-C$_{5'}$*H*), 7.05-7.07 (2H, m, Trp-C$_{2'}$*H* and Trp-C$_{6'}$*H*), 7.32 (1H, ov, Trp-C$_{7'}$*H*), 7.48 (1H, ov, Trp-C$_{4'}$*H*), 7.50-7.53 (1H, br m, Gly-N*H*), 7.63 (1H, ov br m, Leu^3-N*H*), 7.96-7.99 (2H, ov, Trp-N*H* and Leu^4-N*H*), 8.47 (1H, s, Leu^5-N*H*), 8.59 (1H, ov, Thr-N*H*), 10.82 (1H, br s, Trp-N$_{1'}$*H*); ^{13}C NMR (150 MHz, DMSO-d$_6$) δ_C (major conformer) 19.4 (Thr-C$_\gamma$H$_3$), 20.9 (Pro-C$_\gamma$H$_2$), 21.4 (Leu^3-C$_\delta$H$_3$), 22.3 (Leu^5-C$_\delta$H$_3$), 22.7 (Leu^3-C$_{\delta'}$H$_3$), 22.9 (Leu^4-C$_\delta$H$_3$ and Leu^4-C$_{\delta'}$H$_3$), 22.9 (Leu^5-C$_{\delta'}$H$_3$), 24.1 (Leu^5-C$_\gamma$H), 24.2 (Leu^3-C$_\gamma$H), 24.5 (Leu^4-C$_\gamma$H), 27.2 (Trp-C$_\beta$H$_2$), 30.0 (Pro-C$_\beta$H$_2$), 39.1-40.2 (Leu^4-C$_\beta$H$_2$ and Leu^3-C$_\beta$H$_2$), 40.9 (Leu^5-C$_\beta$H$_2$), 42.4 (Gly-C$_\alpha$H$_2$), 45.6 (Pro-C$_\delta$H$_2$), 50.8 (Leu^5-C$_\alpha$H), 51.8 (Leu^4-C$_\alpha$H), 53.7 (Leu^3-C$_\alpha$H), 55.7 (Trp-C$_\alpha$H), 57.6 (Thr-C$_\alpha$H), 60.1 (Pro-C$_\alpha$H), 66.4 (Thr-C$_\beta$H), 110.2 (Trp-C$_{3'}$), 111.3 (Trp-C$_{7'}$H), 117.8 (Trp-C$_{4'}$H), 118.6 (Trp-C$_{5'}$H), 121.0 (Trp-C$_{6'}$H), 123.2 (Trp-C$_{2'}$H), 126.9 (Trp-C$_{3a'}$), 136.2 (Trp-C$_{7a'}$), 168.3 (Thr-*C*O), 169.3 (Gly-*C*O), 170.6 (Pro-*C*O), 171.4 (Trp-*C*O), 171.7 (Leu^4-*C*O), 171.8 (Leu^5-*C*O), 172.1 (Leu^3-*C*O); δ_C (minor conformer) 19.0 (Thr-C$_\gamma$H$_3$), 20.7 (Leu^3-C$_\delta$H$_3$), 21.0 (Leu^3-C$_{\delta'}$H$_3$), 21.4 (Leu^4-C$_\delta$H$_3$), 21.8 (Leu^4-C$_{\delta'}$H$_3$), 22.6 (Leu^5-C$_\delta$H$_3$), 23.1 (Leu^5-C$_{\delta'}$H$_3$), 24.2-24.3 (Leu^3-C$_\gamma$H, Leu^4-C$_\gamma$H, Leu^5-C$_\gamma$H and Pro-C$_\gamma$H$_2$), 26.4 (Trp-C$_\beta$H$_2$), 28.5 (Pro-C$_\beta$H$_2$), 39.1-40.2 (Leu^3-C$_\beta$H$_2$, Leu^4-C$_\beta$H$_2$ and Leu^5-C$_\beta$H$_2$), 42.1 (Gly-C$_\alpha$H$_2$), 47.4 (Pro-C$_\delta$H$_2$), 52.1 (Leu^3-C$_\alpha$H), 52.6 (Leu^5-C$_\alpha$H), 53.5 (Leu^4-C$_\alpha$H), 54.4 (Trp-C$_\alpha$H), 56.2 (Thr-C$_\alpha$H), 60.9 (Pro-C$_\alpha$H), 66.8 (Thr-C$_\beta$H), 110.2 (Trp-C$_{3'}$), 111.4 (Trp-C$_{7'}$H), 117.9 (Trp-C$_{4'}$H), 118.3 (Trp-C$_{5'}$H), 120.9 (Trp-C$_{6'}$H), 123.2 (Trp-C$_{2'}$H), 127.4 (Trp-C$_{3a'}$), 136.1 (Trp-C$_{7a'}$), 168.5 (Gly-*C*O), 170.1 (Thr-*C*O), 170.8 (Pro-*C*O), 170.9 (Trp-*C*O), 171.5 and 171.5 (Leu^5-*C*O and Leu^4-*C*O), 172.4 (Leu^3-*C*O); HRMS (ESI-TOF) *m/z* [M+Na]$^+$ Calcd for $C_{40}H_{60}N_8O_8Na^+$ 803.4426; found 803.4426.

The observed data were consistent with those previously reported.[73]

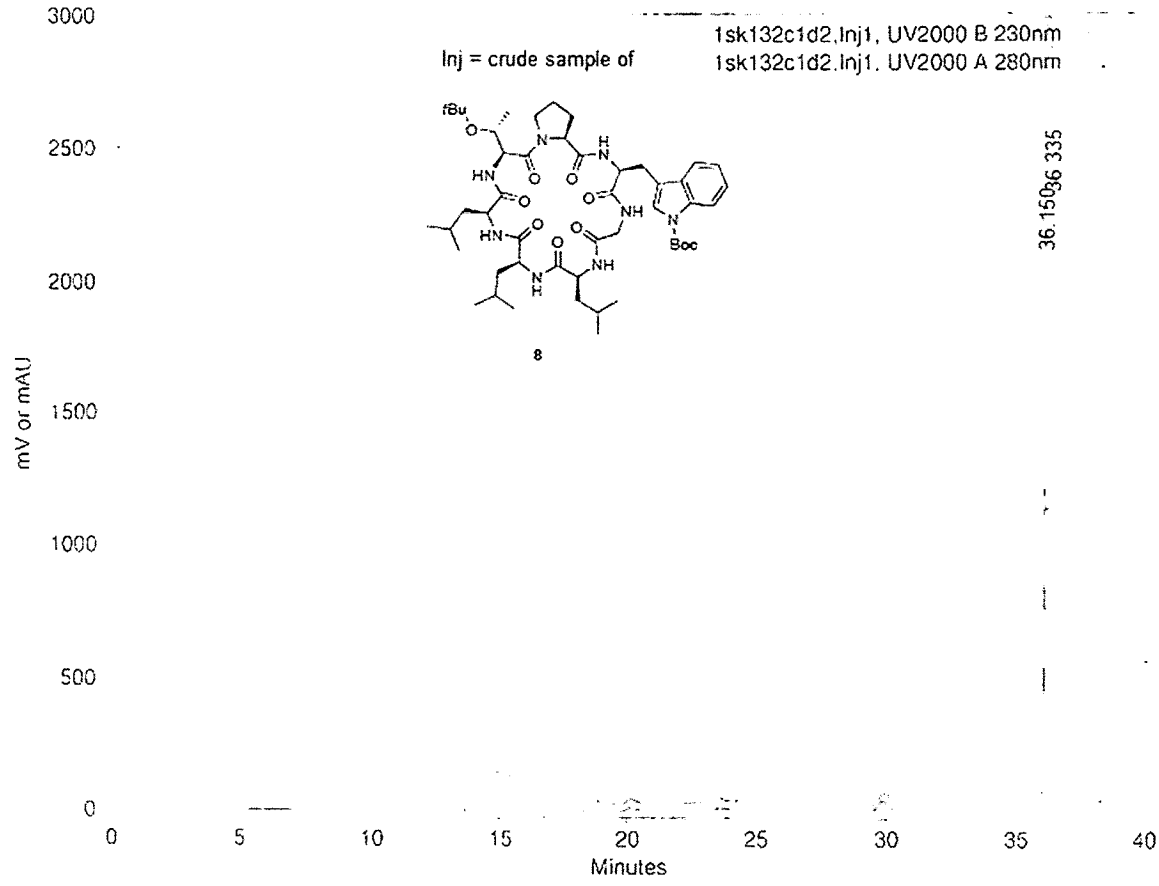
NB: In DMSO-d$_6$, the Pro amide bonds of the major and minor conformers of **40** were found to exhibit the *cis*- and *trans*-configurations, respectively. The following abbreviations are used in the NMR assignments: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; br, broad; app, apparent; ov, overlapped.

It is worth noting that when integerrimide A (**40**) was left as a solution of DMSO-d$_6$ at room temperature for 7 d, it was found to have converted into its oxidised derivatives. After removing DMSO-d$_6$, the resulting residue was subjected to RP-HPLC (10-70% MeCN in H_2O, 40 min, flow rate 11 ml/min, on a Vydac® 218TP™ C18 column, 10 µm, 250 × 22 mm) and the eluates obtained analysed by HRMS. None of *m/z* that corresponded to the [M+Na]$^+$ ions of **40** ($C_{40}H_{60}N_8O_8Na^+$) was observed but the following *m/z* of [M+Na]$^+$ that corresponded to $C_{40}H_{60}N_8O_9Na^+$, $C_{40}H_{60}N_8O_{10}Na^+$ (major), $C_{41}H_{62}N_8O_{10}Na^+$, $C_{40}H_{58}N_8O_9Na^+$, and $C_{40}H_{59}ClN_8O_9Na^+$.

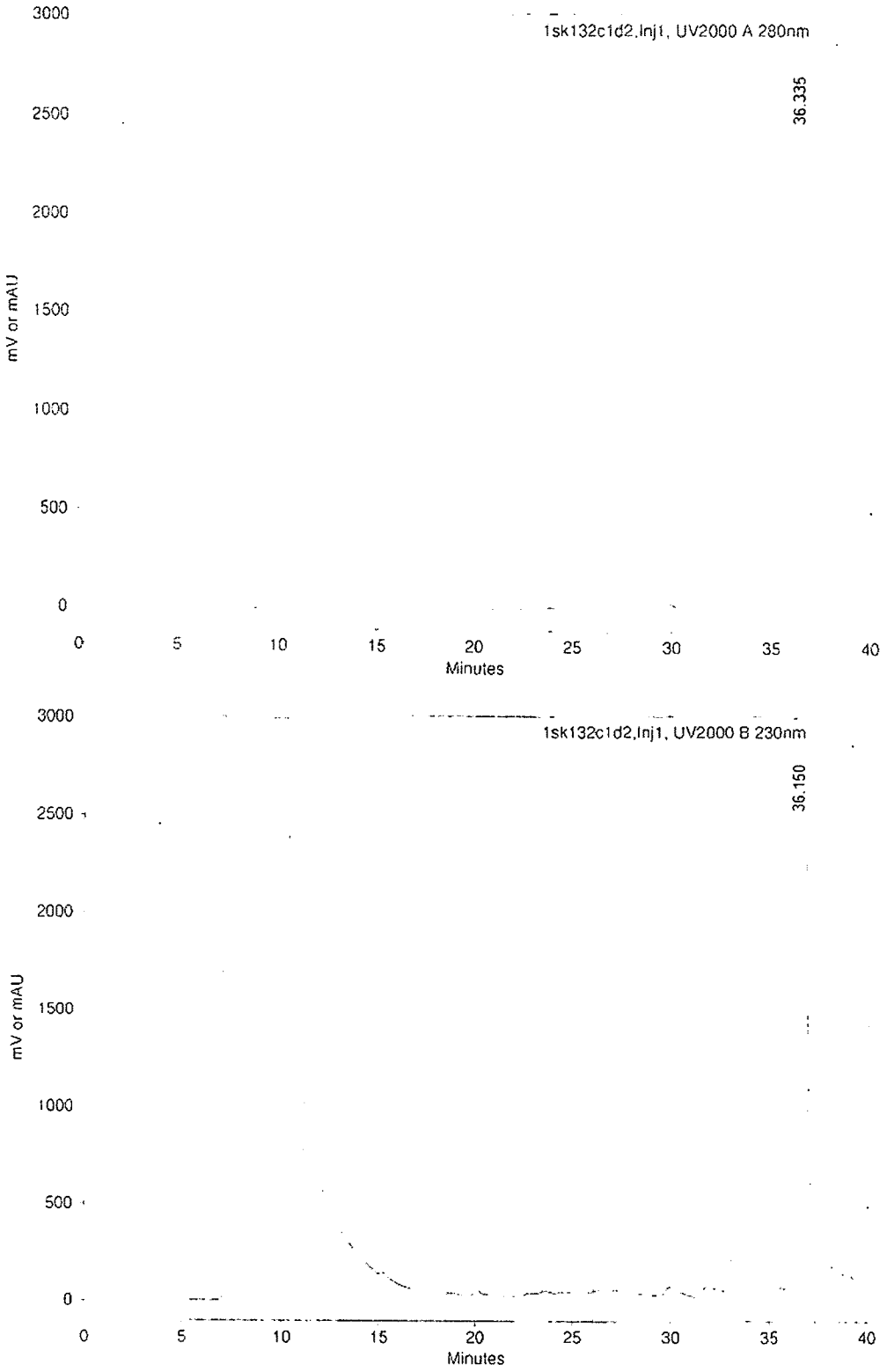
This was likely to be due to irreversible oxidation of the unprotected indole unit of Trp in the natural product.[90]

6.3 HPLC Chromatograms

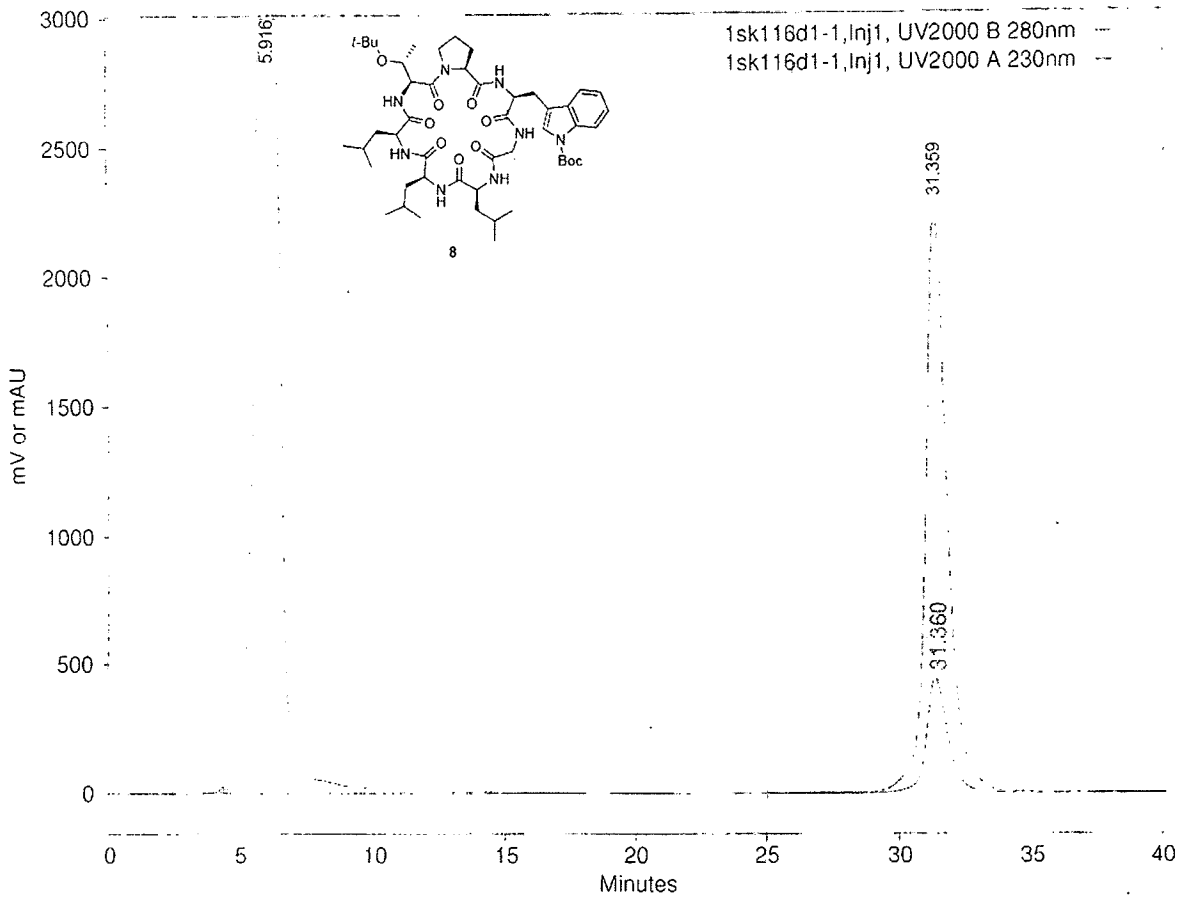
Preparative RP-HPLC of crude material of intermediate **53** (loaded as a solution in DMF): 0-100% MeCN in H_2O, 40 min, flow rate 11 ml/min, on a Vydac® 218TP™ C18 column (10 µm, 250 × 22 mm). t_R = 36 min.



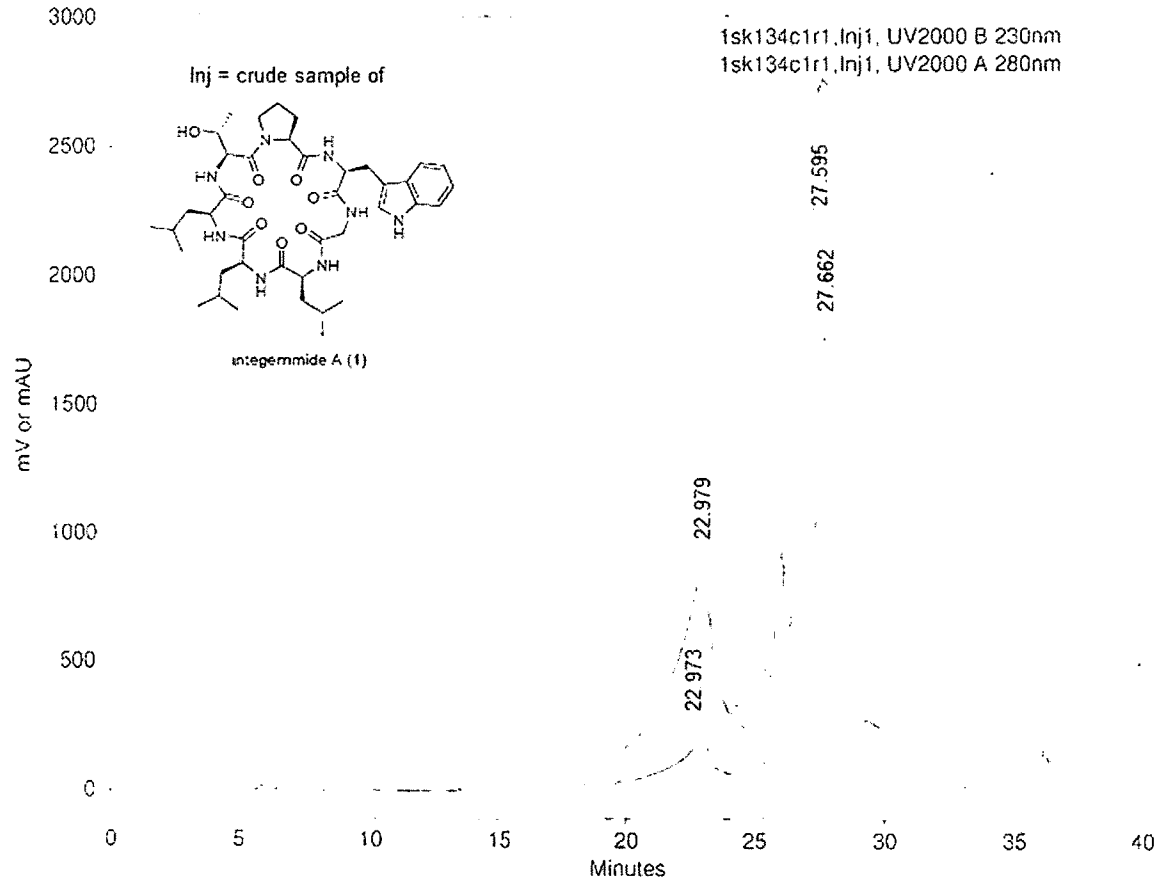
Preparative RP-HPLC of crude material of intermediate **53** (loaded as a solution in DMF): 0-100% MeCN in H_2O, 40 min, flow rate 11 ml/min, on a Vydac® 218TP™ C18 column (10 µm, 250 × 22 mm). t_R = 36 min.



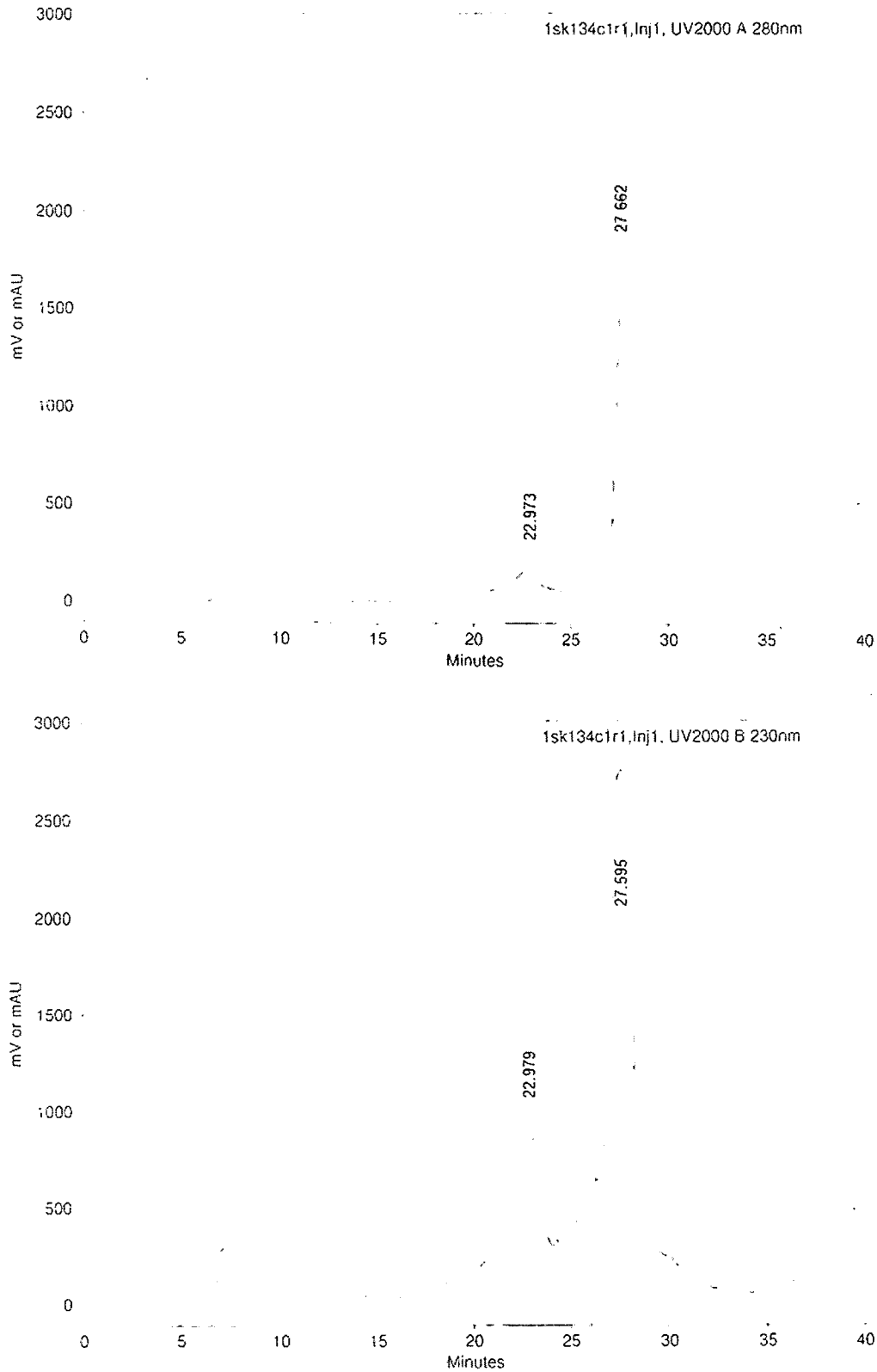
Preparative RP-HPLC of intermediate **53** (loaded as a solution in DMF): 50-70% MeCN in H_2O, 40 min, flow rate 11 mL/min, on a Vydac® 218TP™ C18 column (10 µm, 250 × 22 mm). t_R = 31 min.



Preparative RP-HPLC of crude material of integerrimide A (**40**): 0-100% MeCN in H_2O, 40 min, flow rate 11 ml/min, on a Vydac® 218TP™ C18 column (10 µm, 250 × 22 mm). t_R = 23 and 28 min.



Preparative RP-HPLC of crude material of integerrimide A (**40**): 0-100% MeCN in H_2O, 40 min, flow rate 11 ml/min, on a Vydac® 218TP™ C18 column (10 µm, 250 × 22 mm). t_R = 23 and 28 min.



Preparative RP-HPLC of integerrimide A (**40**): 10-70% MeCN in H_2O, 40 min, flow rate 11 ml/min, on a Vydac® 218TP™ C18 column (10 µm, 250 × 22 mm). t_R = 32 min.