Metal-catalysed tandem metathesis–hydrogenation reactions for the synthesis of carba analogues of cyclic peptides[1]

Amanda N. Whelan, Jomana Elaridi, Roger J. Mulder, Andrea J. Robinson, and W. Roy Jackson

Abstract: Dicarba cyclic peptide analogues of the cystine-containing octapeptides octreotide, lanreotide, and vapreotide, all known somatostatin analogues, have been synthesized via an on-resin homogeneous metal-catalysed sequence involving ruthenium-catalysed ring-closing metathesis followed by rhodium-catalysed hydrogenation.

Key words: dicarba-analogues of cystine-containing peptides, octreotide, vapreotide, and lanreotide; tandem ring-closing metathesis and hydrogenation.

Résumé : Des peptides cycliques dicarbonés analogues de la cystine contenant les octapeptides octréotide, lanréotide et vapréotide, tous connus comme étant des analogues de la somatostatine, ont été synthétisés sur résine via une série de catalyses homogènes impliquant une métathèse de fermeture de cycle catalysée par du ruthénium et une hydrogénation catalysée par du rhodium.

Mots clés : analogues dicarbonés de la cystine contenant des peptides, octréotide, vapréotide et lanréotide; réaction tandem de fermeture de cycle par métathèse et hydrogénation.

Introduction

Cystine bridges are common structural motifs in naturally occurring cyclic peptides. In some cases, as in spiruchostatin A (1), the disulphide bridge acts as a reactive functional group undergoing reduction within the cell to release metal-chelating thiol groups (2, 3). In many other cases, however, the cystine bridge serves only a skeletal, structural role, maintaining secondary and tertiary structure and may be replaced with a nonreducible structural mimic without significantly affecting biological activity (4). Indeed, thioether linkages (-S–CH_2-) (5) and the all carbon (-CH_2–CH_2-) bridge (6) have both been successfully implemented as cystine (-S–S-) isosteres producing peptides with enhanced biostability.

The cyclic peptide octreotide **1a** (7), a synthetic analogue of the tetradecapeptide somatostatin, has one cystine bridge that is remote from the receptor binding site. When labelled with ^{111}In, it is marketed as OctreoScan® (8) and used for the imaging of neuroendocrine tumours (9). It has also been conjugated with ^{123}I, ^{64}Cu, and ^{99m}Tc (10). The ^{99m}Tc radionuclide possesses a convenient physical half life ($t_{1/2}$ = 6 min), an attractive radiation profile, is inexpensive, and readily available as pertechnate ($^{99m}TcO_4^-$).[3] Incorporation of this isotope into cystine-containing peptides, however, is often complicated by the need for a reductive step to generate Tc^{5+} for stable chelate formation (11). In the case of octreotide, a concomitant reduction of the disulphide bridge opens the cyclic peptide and results in the loss of receptor binding affinity (12). This problem is not exclusive to octreotide and also occurs during the Tc labelling of other cyclic peptides containing cystine bridges, including the octreotide analogues lanreotide **2a** and vapreotide **3a**. Given the high desirability to label these peptides with technetium, the replacement of the reductively labile disulphide bridge with a carbon-based manifold has been investigated leading to the dicarba analogues **1b**, **2b**, and **3b**. This structural alteration would be inert to cellular reductases and also with-

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Dedicated to Professor Howard Alper in recognition of his major contribution to metal-catalysed reactions in organic synthesis.

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[3]^{99m}Tc has superior nuclear characteristics for imaging ($t_{1/2}$ = 6.02 h and monoenergetic emission 141 keV), while ^{188}Re physical characteristics are ideal for therapy ($t_{1/2}$ = 16.9 h and β-emission 2.11 MeV).

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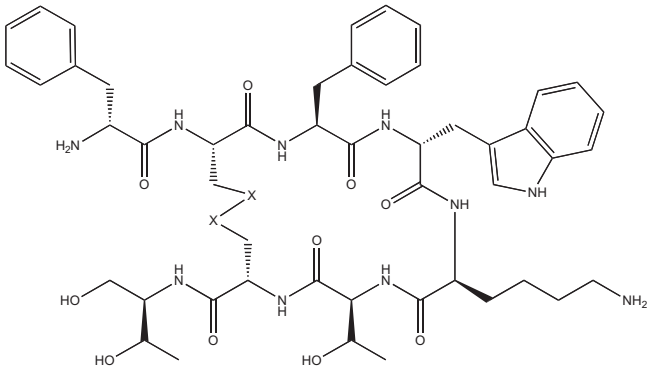
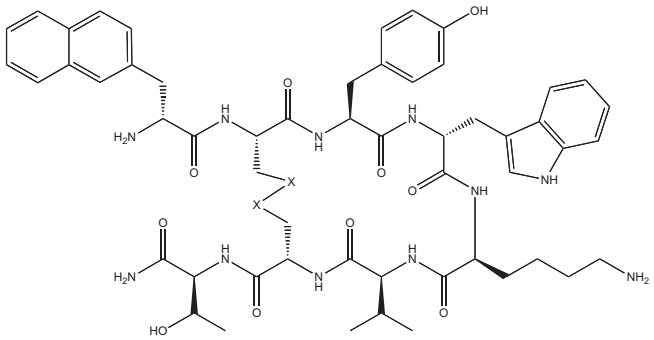
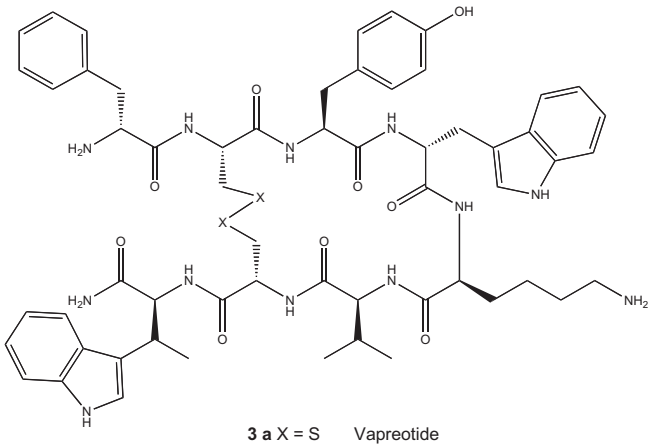


1 a X = S Octreotide
1 b X = CH_2 Dicarba octreotide analogue



2 a X = S Lanreotide
2 b X = CH_2 Dicarba lanreotide analogue

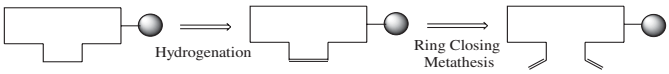


3 a X = S Vapreotide
3 b X = CH_2 Dicarba vapreotide analogue

stand chemical reducing agents, such as stannous chloride, used in the preparation of Tc-labelled radiopharmaceuticals.

A highly generic, on-resin, tandem-homogeneous catalysis strategy has been devised, which involves: (*i*) construction of the linear peptide sequence replacing cysteine residues with allyl glycine; (*ii*) Ru(II)-catalysed metathesis of the allyl groups to affect carbocycle formation; and (*iii*) Rh(I)-catalysed homogeneous reduction of the newly formed olefin to yield the desired sp^3-hybridized bridge (13) (Scheme 1).

Scheme 1.



Grubbs catalysts (14) have previously been used to facilitate the formation of cyclic peptides via metathesis (15), but only Vederas and co-workers (16), in a recent paper targeting oxytocin, have used bis(allyl glycine) containing peptides to form dicarba mimics of the cystine bridge. We believe that the protocol outlined in this paper demonstrates some clear advantages over the previously published method. It is also highly generic and can be applied to any peptide containing a cystine bridge.

Results and discussion

Synthesis of linear peptides

The protocol described utilizes the non-proteinaceous amino acid allyl glycine, which we have previously reported can be conveniently generated in both enantiomeric forms, via Rh(I)-catalysed hydrogenation of α-*N*-acyl dienamides with excellent stereoselectivity (95% ee) and yield (90%) (17). It is also commercially available, albeit at high cost.

The synthesis of dicarba-octreotide analogue **1b** also requires a second non-proteinaceous residue, Fmoc-protected threoninol. This residue is readily formed by the reduction of the protected amino acid threonine to the corresponding alcohol, although it is also commercially available. The resultant diol is functionalized as the *p*-carboxybenzaldehyde acetal in preparation for attachment to the resin.

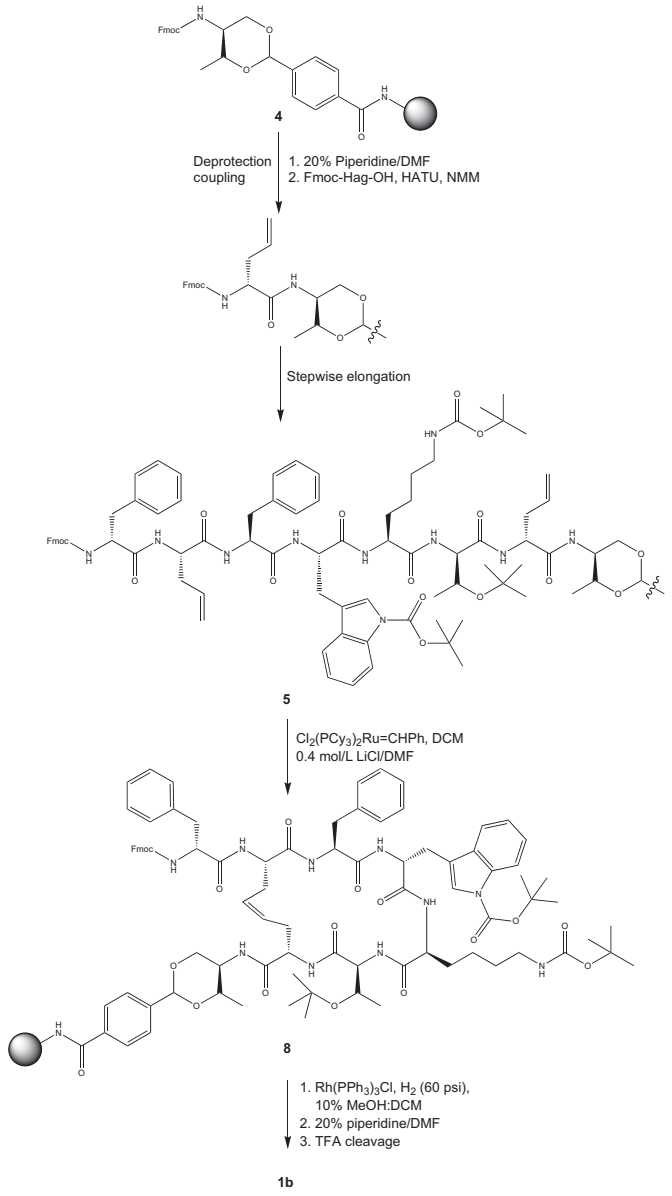
The linear peptide precursor of the dicarba-octreotide analogue was synthesized on Rink amide resin using a modified solid-phase protocol described by Hsieh and co-workers (18). The above described acetal is attached to the resin using the coupling reagents DIC and HOBt to give **4**. The remaining sequence was constructed manually using standard solid-phase peptide synthesis techniques, utilizing Fmoc-protected amino acids and the coupling reagents HATU and NMM (Scheme 2). Intermediates were carried through without purification or characterization up to the octapeptide **5**. A sample of the linear peptide was obtained by cleavage from the resin and was determined to be of >95% purity by reverse-phase analytical chromatography and had the required molecular mass by mass spectral analysis.

The resin-bound linear peptide precursors for the synthesis of dicarba-analogues of lanreotide **2b** and vapreotide **3b** were also prepared on Rink amide resin (Scheme 3). Use of this resin allowed the direct generation of the required C-terminal amide found in **2b** and **3b** upon acid cleavage from the resin. All couplings were accomplished utilizing HATU and NMM. After complete sequence construction, a small sample was cleaved from the resin and the presence of the desired peptide was confirmed by mass spectral analysis.

Cyclisation by ring-closing metathesis of the linear peptides

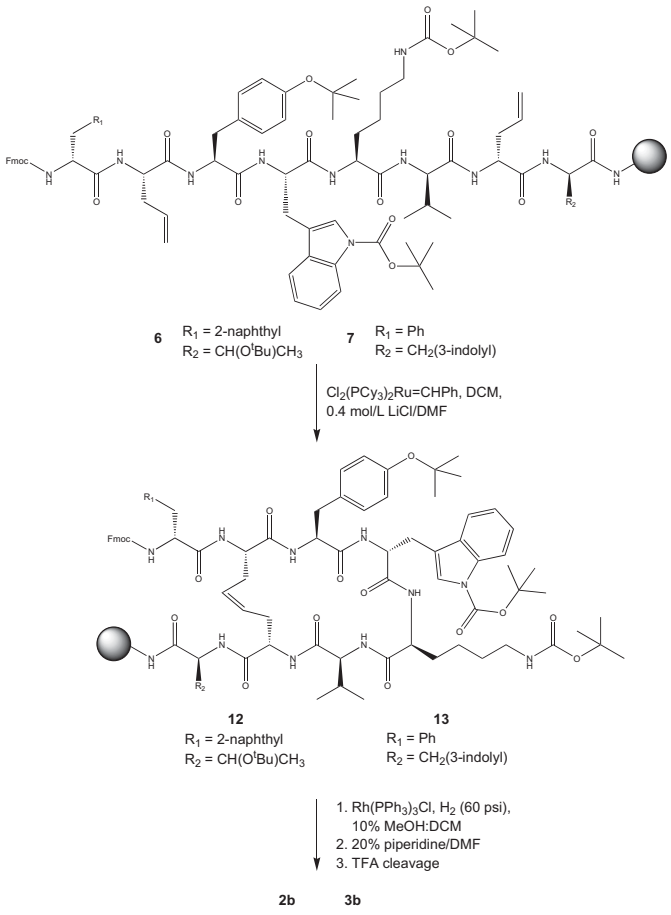
Ring-closing metathesis was carried out using the resin-attached linear peptides **5–7** to eliminate any potential problems arising from dimerization and (or) poor peptide solubility.

Scheme 2.

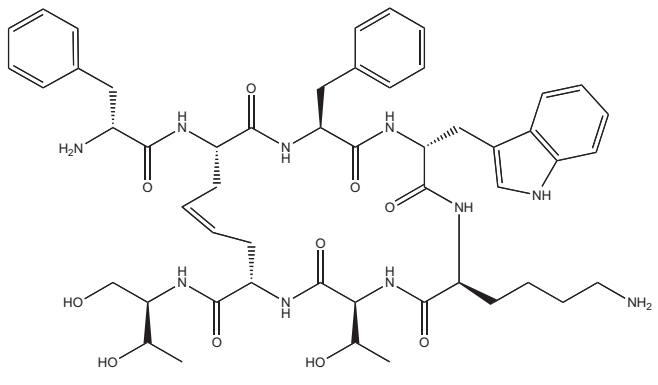


Exposure of the fully protected allyl glycine containing octreotide sequence **5** to the ruthenium benzylidene complex $Cl_2(PCy_3)_2Ru$=CHPh (Grubbs catalyst) (14) resulted in formation of the carbocycle **8** in 65% yield[4] (Scheme 2). It was necessary to react the peptide whilst fully protected as it was found that exposed amino and carboxylate groups prevented metathesis, in agreement with previous observations with other systems (19). To prevent aggregation of the resin-tethered peptide during the metathesis reaction, it was vital that a salt be added, in this case a solution of lithium chloride in DMF. Also essential for complete reaction were extended reaction times; reaction times less than 48 h yielded a significant amount of starting material. Significantly, no oligomerization or dimerization by acyclic diene metathesis (ADMET) (20) was observed.

Scheme 3.



On completion of the metathesis, an aliquot of peptide was cleaved from the resin for analysis. Analytical LC–MS analysis of the cleaved material revealed one major product with molecular mass consistent with that of **9** and a pure sample (>98%) was obtained in 46% yield after purification by preparative HPLC.



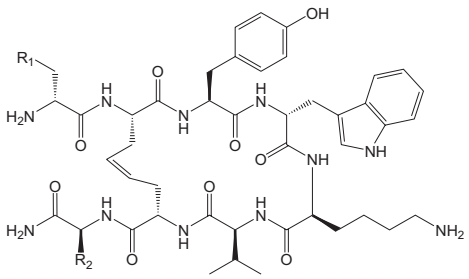
9

The geometry of the newly formed olefinic bond of **9** was investigated by NMR spectroscopy. The olefinic protons are represented in the ^{1}H NMR spectrum by a broad and ill-

[4] Yields of peptides were determined after cleavage of the peptide from the resin and calculated using an average peptide loading of 0.71 mmol/g of resin.

resolved signal, centred on 5.38 ppm, which integrates to two hydrogens. COSY and TOCSY experiments correlated this signal to both methylene groups adjacent to the newly formed carbon–carbon double bond (2.20/2.36 and 2.27 ppm). Whilst coupling constants could not be accurately extracted from the one-dimensional ^{1}H NMR spectrum or from the phase-sensitive COSY spectrum, simulation[5] of the NMR signals using the estimates of the coupling constants from these spectra indicates that the newly formed olefin is cis in geometry. The simulated spectrum that achieved very good similarity with the experimental data was obtained using a vicinal olefinic coupling constant of 10.2 Hz, which is consistent with the cis geometry. Good agreement between the simulated and experimental spectra could not be achieved using values of 14–19 Hz, which would be consistent with a trans double bond.

Cyclisation of the resin-attached dicarba-lanreotide **6** and dicarba-vapreotide **7** precursors proceeded via the same technique as described above (Scheme 3). The reactions did, however, require longer reaction times, with both reactions reaching completion after 96 h. In each case, mass spectral analysis of the Fmoc- and resin-cleaved peptides showed only the desired product and no remaining linear precursor or higher oligomers. Preparative HPLC of the crude unsaturated cyclic peptides **10** and **11** resulted in high purity material albeit at reduced yield. The lanreotide **10** and vapreotide **11** analogues were subsequently isolated in 25% and 2% yield with 98.6% and 99.2% purity, respectively.



10 R_1 = 2-naphthyl, R_2 = CH(OH)CH$_3$ **11** R_1 = Ph, R_2 = CH$_2$(3-indolyl)

Reduction of olefinic dicarba-peptides

Reduction of the newly installed olefinic bond is necessary to complete the synthesis of the dicarba analogues of octreotide **1b**, lanreotide **2b**, and vapreotide **3b**. A homogeneous hydrogenation catalyst, the Rh(I)-based Wilkinson's catalyst, was chosen to facilitate the reduction of the resin-bound material. This catalyst was chosen for its ease of use and facile removal from resin-bound peptides.

Reduction in dichloromethane alone, a solvent preferred for efficient swelling of the Rink amide resin but non-ideal for hydrogenation, was found to be exceptionally slow and often incomplete. The addition of a coordinating solvent subsequently enabled quantitative reduction (21). Traditional hydrogenation solvents such as methanol have very poor resin-swelling abilities, resulting in limited availability of reactive sites. Rh(I)-catalysed hydrogenation in 10% methanol – dichloromethane was investigated and this system was found to effect quantitative reduction at room temperature under mild hydrogen pressure (60 psi, 1 psi = 6.894 757 kPa). The peptide **1b** was purified using reverse-phase preparative chromatography to give material of >98.9% purity in 26% isolated yield and characterized using mass spectrometry and ^{1}H NMR spectroscopy.

Table 1. ^{1}H NMR chemical shifts (ppm) of dicarba-octreotide analogue **1b** (500.13 MHz, DMSO-d_6).

	NH	α-H	β-H	Others
D-Phe[1]	7.59	4.30	2.90, 2.75	H2, H6 7.62
				H3, H5 7.35
				H4 7.27
Hag[2]	8.09	4.29	1.05, 1.05	γ-H 4.05
Phe[3]	7.80	4.61	2.68, 2.80	H2, H6 7.81
				H3, H5 7.60
				H4 7.28
D-Trp[4]	8.49	4.28	3.05, 2.82	H2 8.51
				H4 7.05
				H5 7.50
				H6 7.32
				H7 6.95
Lys[5]	8.23	4.08	1.61, 1.61	γ-H 0.95, 0.95
				δ-H 1.45, 1.45
				ε-H 2.60, 2.60
Thr[6]	7.74	4.02	4.88	γ-H 1.08
Hag[7]	7.66	4.22	1.58	γ-H 4.10
Thr(ol)[8]	7.15	3.58	3.82	γ-H 0.95
				δ-H 2.62

The hydrogenation conditions established for the resin-tethered octreotide analogue were then applied to the olefinic dicarba analogues of lanreotide **12** and vapreotide **13** yielding the saturated carbocycles **2b** and **3b** as indicated by MS and NMR (Scheme 3). Preparative HPLC again gave significantly reduced mass recoveries of highly pure materials, 4% and 20% yields, respectively, for **2b** and **3b**, with purities of >97%.

Molecular modelling studies of carbocycle **1b** (22) based on X-ray diffraction data of octreotide (23) suggests that the structural modification should not significantly perturb the Phe^7-D-Trp^8-Lys^9-Thr^{10} binding domain and hence somatostatin receptor affinity. A detailed NMR study is in progress and ^{1}H chemical shifts for **1b** and its unsaturated analogue **9** are summarized in Tables 1 and 2, respectively. ^{1}H NMR data for the dicarba-lanreotide **2b** is shown in Table 3. The preliminary data suggests the presence of a β-turn, in agreement with the results of molecular modelling studies.

Competitive binding experiments of the six dicarba analogues **1b**–**3b** and **9**–**11** against ^{111}In-DOTA-octreotate for the sst2-expressing AR42J cell membranes is currently being investigated. Further work will involve testing the binding affinity of the new dicarba somatostatin analogues for other somatostatin receptor sub-types and the effect of conjugating bifunctional ligands on their affinity.

[5] Simulation was performed using Bruker-Biospin's NMR-Sim® v3.5. Spectral simulations were performed using the same acquisition and processing parameters that were used to acquire the original data.

Table 2. ^{1}H NMR chemical shifts (ppm) of unsaturated dicarba-octreotide analogue **9** (500.13 MHz, DMSO-d_6).

	NH	α-H	β-H	Others[a]
D-Phe[1]	7.37	4.41	2.71, 3.02	H2, H6
				H3, H5
				H4
Hag[2]	8.43	4.71	2.20, 2.36	γ-H 5.38
Phe[3]	8.05	4.62	2.76, 2.76	H2, H6
				H3, H5
				H4
D-Trp[4]	8.57	4.35	2.79, 3.03	H2
				H4
				H5
				H6
				H7
Lys[5]	8.60	4.02	0.96, 0.96	γ-H 1.42, 1.42
				δ-H 1.70, 1.70
				ε-H 2.63, 2.63
Thr[6]	7.71	4.29	3.99, 3.99	γ-H 1.04
Hag[7]	7.82	4.59	2.27, 2.27	γ-H 5.38
Thr(ol)[8]	7.37	3.97	3.65, 3.65	γ-H 1.07
				δ-H 3.37, 3.49

[a]Signal overlap prohibits unambiguous assignment of aromatic protons.

Table 3. ^{1}H NMR chemical shifts (ppm) of dicarba-lanreotide analogue **2b** (500.13 MHz, DMSO-d_6).

	NH	α-H	β-H	Others
D-β-Nal[1]	7.67	4.43	2.93, 3.12	H1 7.73
				H3 7.43
				H4 7.74
				H5 7.81
				H6 7.38
				H7 7.32
				H8 7.85
Hag[2]	8.12	4.28	1.05, 1.05	γ-H 1.34, 1.34
Tyr[3]	7.81	4.54	2.55, 2.69	H2, H6 6.70, 6.70
				H3, H5 6.48, 6.48
D-Trp[4]	8.38	4.31	2.85, 3.06	H2 7.78
				H4 7.51
				H5 6.97
				H6 7.04
				H7 7.29
Lys[5]	7.92	4.16	1.61, 1.61	γ-H 1.06, 1.06
				δ-H 1.40, 1.40
				ε-H 2.67, 2.67
Val[6]	7.65	3.98	1.96	γ-H 0.88, 0.88
Hag[7]	7.95	4.31	1.05, 1.05	δ-H 1.63, 1.63
Thr[8]	7.41	4.83	4.11	γ-H 0.99

Conclusion

The methodology described in this paper is a highly efficient, generic, on-resin route to carbon-based somatostatin mimics, which could find wide-spread use in peptidomimetic research. Our group is currently applying this methodology to other peptides, particularly cystine-containing cyclic peptides.

Experimental

Instrumentation and general procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX300 spectrometer operating at 300.13 MHz (^{1}H) and 75.48 MHz (^{13}C).

The NMR spectra of the peptides were recorded in DMSO-d_6 (referenced to δ_H 2.49 ppm) at 298 K using a Bruker DRX500 spectrometer at 500.13 MHz for ^{1}H using a 5 mm inverse-detection ^{1}H–^{13}C–^{15}N TXI probe with z gradients. Pulse sequences are from the Bruker XWIN-NMR v3.5 library. Chemical shift data were derived from a combination of the COSY45, COSY, and TOCSY experiments. Assignments were made using standard peptide NMR assignment techniques (24).

The two-dimensional COSY45 experiments were performed in magnitude mode using gradient selection and a 45° pulse: 4096 points in t_2 and 512 points in t_1 were acquired with 16 scans per t_1 point and a 0.8 s relaxation delay between scans. The spectral width was 6000 Hz in both dimensions. 2048 Points in t_2 and zero-filling to 1024 points in t_1 were used. An unshifted sine-bell window function was applied in both dimensions prior to Fourier transformation.

The two-dimensional COSY experiments were performed in phase-sensitive mode using echo/antiecho–TPPI gradient selection: 4096 points in t_2 and 512 points in t_1 were acquired with 64 scans per t_1 point and a 0.8 s relaxation delay between scans. The spectral width was 6000 Hz in both dimensions. Zero-filling to 4096 points in t_2 and forward linear prediction to 1024 points and zero-filling to 4096 points in t_1 were used. An unshifted sine-bell window function was applied in both dimensions prior to Fourier transformation.

The two-dimensional TOCSY experiments were performed in phase-sensitive mode using echo/antiecho–TPPI gradient selection: 4096 points in t_2 and 512 points in t_1 were acquired with 32 scans per t_1 point and a 0.8 s relaxation delay between scans. The spectral width was 6000 Hz in both dimensions. The TOCSY mixing time was 120 ms. 2048 Points in t_2 and zero-filling to 1024 points in t_1 were used. A π/4-shifted, sine-squared-bell window function was applied in both dimensions prior to Fourier transformation.

The two-dimensional NOESY experiments were performed in phase-sensitive mode using States gradient selection: 4096 points in t_2 and 512 points in t_1 were acquired with 64 scans per t_1 point and a 0.8 s relaxation delay between scans. The spectral width was 6000 Hz in both dimensions. The NOESY mixing time was 400 ms. 2048 Points in t_2 and zero-filling to 1024 points in t_1 were used. An exponential window function of 3.0 Hz was applied in the t_2 dimension and a π/4-shifted, sine-squared-bell window function was applied in the t_1 dimension prior to Fourier transformation.

Positive electrospray mass spectral data were obtained on a VG Platform mass spectrometer using a cone voltage of 50 V.

Analytical HPLC spectra were recorded on a Waters Alliance 2695 Separations Module equipped with a Waters PDA 2996 detector monitoring 190–300 nm. Components were separated on either an Altima 150 mm × 4.6 mm C18 5 μm

Table 4. Data for cyclic peptide analogues.

No.	Molecular formula	Exact mass	*m/z* (Pos. ion) obtained	Retention time (min)[a]	% Yield	% Purity
9	$C_{51}H_{68}N_{10}O_{10}$	980.51	981.2	30.10	46	98.9
1b	$C_{51}H_{70}N_{10}O_{10}$	982.53	983.6	33.37	23	98.8
10	$C_{56}H_{70}N_{11}O_{10}$	1057.54	1058.6	46.00	25	98.6
2b	$C_{56}H_{72}N_{11}O_{10}$	1059.55	1060.3	43.25	4	97.0
11	$C_{59}H_{71}N_{11}O_{10}$	1093.54	1094.5	11.42	2	99.2
3b	$C_{59}H_{73}N_{11}O_{10}$	1095.55	1096.4	39.50	20	99.6

[a]Preparative HPLC.

packed column or a Waters 50 mm × 4.6 mm Xterra C18 2.5 μm packed column. Purification via preparative HPLC was achieved using a Shimadzu LC-4A with an SPD-2AS detector (227 nm) and a C18 Altima 250 mm × 22 nm 5 μm packed column.

Solvents were purified, dried, and degassed according to standard procedures. Degassed methanol and dichloromethane were used in hydrogenation reactions. Deuterated chloroform was used as supplied by Merck. Deuterated dimethylsulphoxide (99.96% D, DLM-34) was used as supplied by Cambridge Isotope Laboratories. Chemical reagents, including amino acids, were purchased from Sigma-Aldrich, AusPep, or GL Biochem and were used without further purification. Catalysts were used as received from Strem chemicals. Grubbs catalyst refers to bis(tricyclohexylphosphine)benzylidene ruthenium dichloride. Wilkinson's catalyst refers to chlorotris(triphenylphosphine)rhodium(I). All ruthenium-catalysed metathesis reactions were performed using standard Schlenk techniques under an atmosphere of nitrogen. In all rhodium-phosphine hydrogenations, high purity (<10 ppm of oxygen) hydrogen and argon (supplied by BOC gases) were used and purified by passage through a series of traps to remove water, oxygen, and hydrocarbons.

Synthesis of the linear peptides

Fmoc-L-threoninol p-*carboxybenzacetal*

Fmoc-threoninol (1.0 g, 3.05 mmol), *p*-carboxybenzaldehyde (0.5 g, 3.36 mmol), and a catalytic amount of *p*-toluenesulphonic acid were suspended in dry toluene (70 mL) and stirred at reflux for 20 h using a Dean–Stark apparatus. The solvent was removed under reduced pressure to yield a clear yellow oil, which was extracted into dichloromethane and washed with water and saturated sodium chloride and dried over sodium sulphate. Purification of the yellow oil by flash chromatography (SiO_2, dichloromethane – ethyl acetate, 3:1) yielded the desired acetal (0.71 g, 51%), mp 86–88 °C (lit. value (18) mp 86–88 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (d, *J* = 6.4 Hz, 3H, C*H*$_3$), 3.73 (dd, *J* = 10.1, 1.6 Hz, 1H, NHCHC*H*(H)), 4.11–4.27 (m, 4H, C*H*CH$_3$, C*H*$_2$OCO, and NHCHCH(*H*)), 4.51–4.38 (m, 2H, H9 and NHC*H*), 5.57 (d, *J* = 9.9 Hz, 1H, N*H*), 5.65 (s, 1H, OC*H*O), 7.32 (t, *J* = 7.4 Hz, 2H, H2, H7), 7.40 (t, *J* = 7.6 Hz, 2H, H3, H6), 7.62 (d, 2H, *J* = 7.2 Hz, H2′, H6′), 7.63 (d, *J* = 8.4 Hz, 2H, H3′, H5′), 7.77 (d, *J* = 7.3 Hz, 2H, H1, H8), 8.14 (d, *J* = 8.3 Hz, 2H, H4, H5), 10.05 (s, 1H, COO*H*). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 17.78 (*C*H$_3$), 47.45 (C9), 48.90 (NH*C*H), 67.12 (NHCH*C*H$_2$), 72.00 (*C*H$_2$OCO), 75.61 (CHCH$_3$), 100.84 (O*C*HO), 120.07 (C3, C6), 124.74 (C2′, C6′), 126.21 (C2, C7), 127.11 (C4, C5), 127.77 (C1, C8), 129.83 (C4′), 130.30 (C3′, C5′), 141.31 (C4a, C4b), 143.82 (C1′), 143.85 (C8a, C9a), 156.52 (*C*O), 170.94 (*C*OOH). MS (ESI$^+$, DCM–MeOH) *m/z*: 350.2 (M + Na$^+$), $C_{19}H_{21}NO_4Na$.

Peptide synthesis

Linear peptides were synthesized using manual solid-phase peptide synthesis techniques on Rink amide resin, utilizing 3 equiv. each of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acids and the coupling reagent HATU (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) and 6 equiv. of NMM (4-methyl morpholine). Fmoc-L-threoninol *p*-carboxybenzacetal was coupled directly to the resin in the synthesis of **5** using 3 equiv. each of the coupling reagents DIC (diisopropylcarbodiimide) and HOBt (1-hydroxybenzotriazole), as well as 3 equiv. of the acetal.

Upon completion of the synthesis of the linear Fmoc-protected octapeptide, a resin aliquot was removed and the peptide cleaved from the resin with trifluoroacetic acid (9 mL) containing the scavengers ethanedithiol (0.25 mL), thioanisole (0.5 mL), and phenol (0.79 g), as well as water (0.25 mL). The filtrate was concentrated by application of high pressure air. The peptide was precipitated by the addition of ice cold diethyl ether and was extracted into a 50% aqueous solution of acetonitrile and lyophilized to give a white solid that was characterized by mass spectral analysis.

General procedure for ring-closing metathesis

A Schlenk flask was charged with resin-tethered peptide (50–200 mg) to which dry dichloromethane (20 mL) and degassed 0.4 mol/L lithium chloride in dimethylformamide (1 mL) were added and the resin allowed to swell for 1 h before addition of a solution of Grubbs catalyst (20 mol%) in dry dichloromethane. The suspended resin was stirred at 50 °C for a prescribed period of time (48–96 h) and collected via filtration, washed with dichloromethane, dimethylformamide, and methanol, and finally dried in vacuo. An aliquot of the resin was removed and the peptide cleaved from the resin as described previously.

The peptide was purified using preparative reverse-phase high performance liquid chromatography (RP-HPLC) (20%–100% aqueous acetonitrile with 0.1% TFA over 2 h, 10 mL/min). Fractions were collected, lyophilized, and analysed by analytical HPLC, NMR, and mass spectral analysis (Table 4).

General procedure for hydrogenation

A Fischer–Porter tube was charged in a drybox with resin-tethered peptide (50–200 mg), Wilkinson's catalyst (1–5 mg), dry and deoxygenated dichloromethane (9 mL), and dry and deoxygenated methanol (1 mL). The line and reaction vessel were degassed with three vacuum–argon cycles each before the tube was pressurised to 60 psi of hydrogen. The reaction was stirred gently at room temperature overnight. The resin was collected via filtration and washed with dichloromethane, dimethylformamide, and finally methanol, before being allowed to dry in vacuo. An aliquot of the resin was removed and the peptide cleaved from the resin as described previously.

The peptide was purified using preparative reverse-phase HPLC (20%–100% aqueous acetonitrile with 0.1% TFA over 2 h, 10 mL/min). Individual fractions were collected, lyophilized, and analysed by analytical HPLC, NMR, and mass spectral analysis (Table 4). ^{1}H NMR data for dicarba octreotide **1b**, unsaturated dicarba octreotide **9**, and dicarba lanreotide **2b** are reported in Tables 1–3.

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References

1. K. Shin-ya, Y. Masuoka, A. Nagai, K. Furihata, K. Nagai, K. Suzuki, Y. Hayakawa, and Y. Seto. Tetrahedron Lett. **42**, 41 (2001).
2. A. Yurek-George, F. Habens, M. Brimmell, G. Packham, and A. Ganesan. J. Am. Chem. Soc. **126**, 1030 (2004).
3. R. Furumai, A. Matsuyama, N. Kobashi, K.-H. Lee, N. Nishiyama, H. Nakajima, A. Tanaka, Y. Komatsu, N. Nishino, M. Yoshida, and S. Horinouchi. Cancer Res. **62**, 4916 (2002).
4. P. Grieco, P. Campiglia, I. Gomez-Monterrey, T. Lama, and E. Novellino. Synlett, **14**, 2216 (2003), and refs. cited therein.
5. (*a*) P. Campiglia, I. Gomez-Monterrey, L. Longobardo, T. Lama, E. Novellino, and P. Grieco. Tetrahedron Lett. **45**, 1453 (2004), and refs. cited therein; (*b*) X. Zhu and R.R. Schmidt. Eur. J. Org. Chem. 4069 (2003), and refs. cited therein.
6. (*a*) R.F. Nutt, R.G. Strachan, D.F. Veber, and F.W.J. Holly. J. Org. Chem. **45**, 3078 (1980); (*b*) K. Undheim, J. Efskind, and G. Beate Hoven. Pure Appl. Chem. **75**, 279 (2003).
7. W. Bauer, U. Briner, W. Doepfner, R. Haller, R. Huguenin, P. Marbach, T.J. Petcher, and J. Pless. J. Life Sci. **31**, 1133 (1982).
8. D.A. Pearson, J. Lister-James, W.J. McBride, D.M. Wilson, L.J. Martel, E.R. Civitello, J.E. Taylor, B.R. Moyer, and R.T. Dean. J. Med. Chem. **39**, 1361 (1996).
9. (*a*) E.P. Krenning, D.J. Kwekkeboom, W.H. Bakker, W.A.P. Breeman, P.M. Kooji, H.Y. Oei, M. van Hagen, P.T.E. Postema, M. de Jong, J.C. Reubi, T.J. Visser, A.E.M. Reijs, L.J. Hofland, J.W. Koper, and S.W. Lamberts. Eur. J. Nucl. Med. **20**, 716 (1993); (*b*) W.H. Bakker, R. Albert, C. Bruns, W.A.P. Breeman, L.J. Hofland, P. Marbach, J. Pless, D. Pralet, B. Stolz, J.W. Koper, S.W.J. Lamberts, T.J. Visser, and E.P. Krenning. Life Sci. **49**, 1583 (1991).
10. J.S. Lewis, M.R. Lewis, A. Srinivasan, M.A. Schmidt, J. Wang, and C.J.J. Anderson. J. Med. Chem. **42**, 1341 (1999).
11. (*a*) T. Maina, B. Nock, A. Nikolopoulou, P. Sotiriou, G. Loudos, D. Maintas, P. Cordopatis, and E. Choitellis. Eur. J. Nucl. Med. **29**, 742 (2002); (*b*) J. Fichna and A. Janecka. Bioconjugate Chem. **14**, 3 (2003).
12. (*a*) H. Kolan, J. Li, and M.L. Thakur. Peptide Res. **9**, 144 (1996); (*b*) J.K. Amartey. Nucl. Med. Biol. **20**, 539 (1993).
13. A.N. Whelan, J. Elaridi, M. Harte, S.V. Smith, W.R. Jackson, and A.J. Robinson. Tetrahedron Lett. **45**, 9545 (2004).
14. S.T. Nguyen, L.K. Johnson, R.H. Grubbs, and J.W. Ziller. J. Am. Chem. Soc. **114**, 9858 (1992).
15. (*a*) H. Kessler and N. Schmiedeberg. Org. Lett. **4**, 59 (2002); (*b*) J.F. Reichwein, C. Versuluis, and R.M.J. Liskamp. J. Org. Chem. **65**, 6187 (2000); (*c*) T.D. Clark and M.R. Ghadiri. J. Am. Chem Soc. **117**, 12 364 (1995); (*d*) S.J. Miller and R.H. Grubbs. J. Am. Chem. Soc. **117**, 5855 (1995); (*e*) E.N. Prabhakaran, V. Rajesh, S. Dubey, and J. Iqbal. Tetrahedron Lett. **42**, 339 (2001); (*f*) H.E. Blackwell, J.D. Sadowsky, R.J. Howard, J.N. Samson, J.A. Chao, W.E. Steinmetz, D.J. O'Leary, and R.H. Grubbs. J. Org. Chem. **66**, 5291 (2001).
16. J.L. Stymiest, B.F. Mitchell, S. Wong, and J.C. Vederas. Org. Lett. **5**, 47 (2003).
17. E. Teoh, E.M. Campi, W.R. Jackson, and A.J. Robinson. Chem. Commun. 978 (2002).
18. H.P. Hsieh, Y.T. Wu, S.T. Chen, and K.T. Wang. Bioorg. Med. Chem. **7**, 1797 (1999).
19. Intolerance of catalysts to basic functionality has been previously reported. (*a*) S. Kotha, N. Sreenivasachary, K. Mohanraja, and S. Durani. Bioorg. Med. Chem. Lett. **11**, 1421 (2001); (*b*) R.H. Grubbs and S. Chang. Tetrahedron, **54**, 4413 (1998); (*c*) T.M. Trnka and R.H. Grubbs. Acc. Chem. Res. **34**, 18 (2001); (*d*) M.S. Sanford, J.A. Love, and R.H. Grubbs. J. Am. Chem. Soc. **123**, 6543 (2003).
20. M.R. Buchmeiser. Chem. Rev. **100**, 1565 (2000).
21. R. Santini, M.C. Griffith, and M. Qi. Tetrahedron Lett. **39**, 8951 (1998). Incompatible resin–solvent pairing can lead to poor reaction site availability and diminished reaction rates. Rink amide resin swells poorly in methanol, but exhibits high swell in chlorinated solvents.
22. The unit cell of octreotide reveals the flexibility of the peptide backbone and contains three individual molecules of the peptide. Only one molecule (Molecule II) possesses the rotomer conformation believed to be associated with biological activity. This molecule was modelled using Insight II with the Discover Minimization Module [computer program]. Vol. 2002. Accelerys, San Diego, Calif. 1996.
23. E. Pohl, A. Heine, and G.M. Sheldrick. Acta Crystallogr. Sect. D, **D51**, 48 (1995).
24. K. Wüthrich. NMR of proteins and nucleic acids. John Wiley and Sons, New York. 1986.