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Efficient Large-Scale Synthesis of CAT811, a Potent Calpain Inhibitor of Interest in the Treatment of Cataracts

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A high-yielding, short, and scalable synthesis of a potent calpain 2 inhibitor (CAT811) is reported. The key step in the sequence involves an intramolecular macrocyclization of a 6-iodonorleucine residue to the side chain of tyrosine.

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Introduction

We recently reported[1] a series of macrocycles as potent and selective inhibitors of calpain 2, the over-activity of which is central to several important disease states.[2] One of these inhibitors, CAT811 **1** (Fig. 1), has shown significant promise in the treatment of cortical cataract.

CAT811 and other members of this class of macrocycle were developed[1] to constrain the constituent peptide backbone into a bioactive β-strand conformation known to favour binding to proteases.[3] The constituent lipophilic constraint was introduced by a ring-closing metathesis (RCM)-based macrocyclization of the P1 and P3[4] allyl substituents of an acyclic precursor, followed by hydrogenation of the alkene.[1] However, this methodology is not suitable for multigramme-scale synthesis as it is moderate-yielding and requires extensive use of chromatography. Despite recent reports of industrial-scale RCM reactions,[5] this step also remains an expensive option. In addition, the original route employs flammable reducing agents and solvents that can form peroxides; again, this is undesirable for a scale-up synthesis. In the present paper, we report (i) a sequence that negates the need for chromatography at all stages in the synthesis; (ii) key closure of the macrocycle via an intramolecular nucleophilic substitution;[6] and (iii) alternative conditions for a key reduction step.

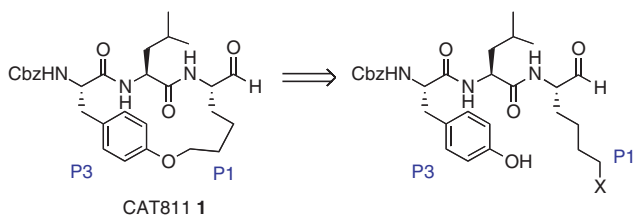

Fig. 1. Retro-synthetic analysis of CAT811 **1**.

Results and Discussion

A retrosynthetic analysis of **1** (as shown in Fig. 1) suggests the key intermediate to be a 'tripeptide' with a tyrosine at P3 and a residue at P1 containing a terminal side-chain electrophile (X) (see **10** for a specific example). With this in mind, we set about a synthesis of compounds **9** and **10** as depicted in Scheme 1. An EDCI (1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride)-catalyzed coupling of Cbz-Tyr-OH **2** with Leu-OMe hydrochloride **3** in the presence of 1-hydroxy-7-azabenzotriazole (HOAt) gave dipeptide **4**. Hydrolysis of the methyl ester of **4** with LiOH in methanol and water gave dipeptide **5** in 96% yield. The hydrochloride salt **7** was prepared (for coupling with **5**) by simultaneous *N*-deprotection and esterification of Boc–Nle(6-hydroxy)–OH **6**[7] with thionyl chloride in the presence of methanol. The dipeptide **5** was then coupled with **7** in the presence of EDCI and HOAt to give the tripeptide **8**, which was converted to the bromide **9** on a small scale (250 mg) on treatment with triphenylphosphine (PPh_3), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and tetrabutylammonium bromide (TBAB) according to the method of Iranpoor.[8] Conversion of **9** to the iodide **10** was achieved quantitatively on treatment with NaI in acetone. For larger-scale synthesis of **10** (>5 g), it proved more efficient to iodinate tripeptide **8** directly using tetrabutylammonium iodide (TBAI), 2,3-dichloro-5,6-dicyano-1,4-benzoquin2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and PPh_3 (Scheme 1). The triphenylphosphine oxide by-product was simply removed from the desired iodide on recrystallization from ethyl acetate and pentane.

The conversion of iodide **10** to macrocycle **11** was first attempted on a small scale (100 mg) on treatment with *N*,*N*-diisopropylethylamine (DIPEA) as the base in dichloromethane (DCM); however, only starting material was isolated after 16-h reaction. Several alternative conditions for the preparation of **11** were investigated, with the optimum proving to be treatment of

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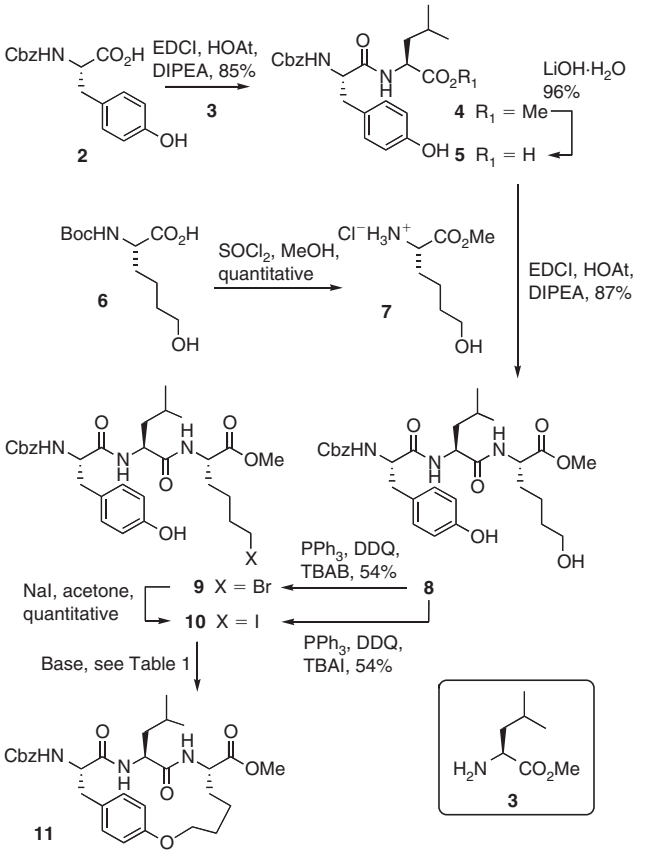


Scheme 1.

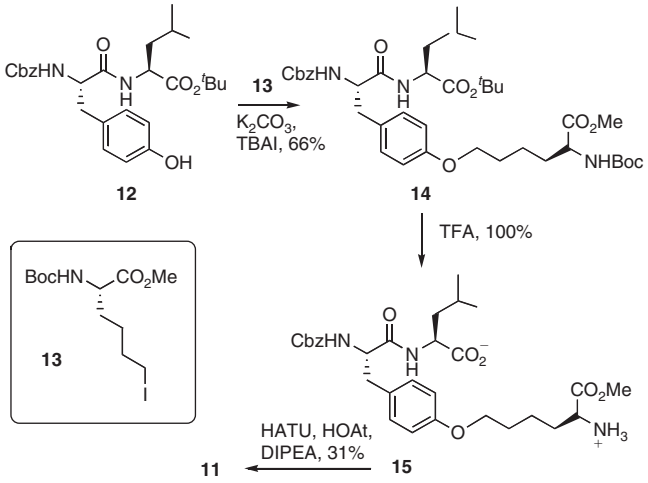
Table 1. Conditions for the macrocyclization of 10

Base	Solvent	Temperature/Time	Yield [%]
K_2CO_3	Toluene	Reflux/16 h	64
K_2CO_3	DMF	Reflux/16 h	67
K_2CO_3	MeCN	Reflux/16 h	68
K_2CO_3/Cs_2CO_3	MeCN	Reflux/16 h	75

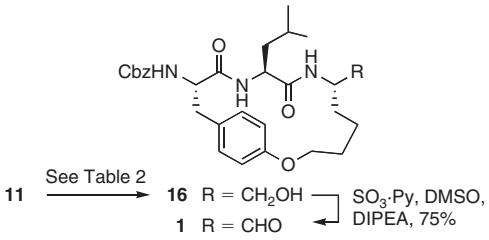
10 with K_2CO_3 in the presence of a catalytic quantity of Cs_2CO_3 (Table 1). A large-scale (7 g) reaction under these conditions gave the desired macrocycle **11** in 70% yield after purification by recrystallization from ethyl acetate and pentane.

An alternative route to **11**, in which the key sequence of reactions in Scheme 1 was reversed, was also investigated to see if there was any advantage in overall yield (Scheme 2). In particular, intermolecular nucleophilic substitution of dipeptide **12** and (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-6-iodohexanoate **13**, synthesized according to the method of Adamczyk,[9] gave **14** in 66% yield. The *C*- and *N*-termini of **14** were then simultaneously deprotected by treatment with trifluoroacetic acid to give **15**, which was cyclized to give macrocycle **11** in 31% yield as shown in Scheme 2. This route was not explored further owing to the low yield of **11**.

The final step in the synthesis of CAT811 **1** (Scheme 3) required the reduction of the *C*-terminal ester of **11** to an aldehyde. Our original small-scale synthesis of **1** achieved this by reduction with $LiAlH_4$ in THF to give the alcohol **16**, which was oxidized with SO_3·pyridine complex in the presence of DMSO to give CAT811.[1]



Scheme 2.



Scheme 3.

Table 2. Conditions for the reduction of 11

Reducing agent	Solvent and conditions	Additive	Yield [%]
$NaBH_4$	THF	$AlCl_3$	9
$NaBH_4$	THF	$ZnCl_2$	36
$NaBH_4$	THF	LiCl	90
$LiBH_4$	THF	–	97
$LiBH_4$	THF	$(MeO)_3B$	98
$LiBH_4$	THF	MeOH	99
$LiBH_4$	Methyl *tert*-butyl ether	MeOH	99

This sequence proved successful on a 100–200 mg scale but lacked reproducibility on a small scale (approx. 10 mg) and furthermore, use of $LiAlH_4$ in THF for multigramme syntheses was deemed unsuitable for a scale-up synthesis. The reduction was thus attempted using $NaBH_4$ in the presence of several additives[10] (Table 2). A key observation was that reduction with $NaBH_4$ in the presence of LiCl gave the desired alcohol in 90% yield (Table 2, entry 3). Based on this observation, the reduction of **11** was attempted on a large scale using $LiBH_4$ in THF, which gave a 97% yield of the desired alcohol **16** after a single recrystallization. The use of $LiBH_4$ in combination with $(MeO)_3B$ or MeOH led to a small improvement in the yield of **16** (98 and 99%, respectively), but this was not considered to offer any significant advantage. Having established $LiBH_4$ as a suitable reducing agent, attention was directed at replacing THF with a more industrially 'friendly' solvent. The use of lithium borohydride in combination with methanol, using methyl *tert*-butyl ether (MTBE, which does not form oxidizable peroxides) as solvent, proved to be a suitable combination that gave an almost quantitative yield of alcohol **16**. The alcohol **16** was then reliably oxidized to aldehyde **1** on multigramme scale-up using SO_3·pyridine in the presence of DMSO (Scheme 3).

Conclusions

Here, we report a high-yielding, short, and scalable synthesis of CAT811 **1**. The synthesis uses non-flammable solvents and can be safely and efficiently carried out on a multigramme scale without the need for chromatography. The key step in the sequence (**10**–**11**) involves the macrocyclization of a 6-iodonorleucine residue to the side chain of tyrosine.

Experimental

Methods and Materials

^{1}H NMR spectra were recorded on an Inova 500 spectrometer operating at 500 MHz unless otherwise stated. Carbon NMR spectra were obtained on a Varian Unity XL 300 MHz Fourier Transform spectrometer operating at 75 MHz. Chemical shifts are reported in parts per million (ppm, δ) referenced relative to the residual solvent peak; coupling constants are reported in Hz.

Electrospray ionization (ESI) mass spectra were detected on a micromass liquid chromatography time of flight mass spectrometer, with a probe voltage of 3200 V, temperature of 150°C, and a source temperature of 80°C. Direct ionization used 10 μL of a 10 μg mL^{-1} solution, using a carrier solvent of 50% acetonitrile/water at a flow rate of 20 μL min^{-1}. Ionization was assisted by the addition of 0.5% formic acid. Melting points were obtained on an electrothermal melting point apparatus and are uncalibrated. TLC was performed on aluminium-backed Merck Kieselgel KG60F$_{254}$ silica plates. Amino acids were purchased from GL Biochem (Shanghai) Limited. Reagents and other chemicals were purchased from Aldrich Chemicals.

((7S,10S,13S)-7-Formyl-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13-yl)-carbamic Acid Benzyl Ester ***1***

The macrocyclic alcohol **16** (2.0 g, 3.8 mmol) was dissolved in a mixture of DCM (20 mL) and DMSO (8 mL) and the solution cooled in an ice-bath. DIPEA (2.65 mL, 15.2 mmol) was added and the solution was stirred for 5 min before the addition of a warmed solution (approx 40°C) of SO_3·pyridine complex (2.42 g, 15.2 mmol) in DMSO (6 mL). The reaction mixture was stirred for 3 h, allowed to warm to room temperature, and then it was diluted with EtOAc (250 mL). The organic phase was separated and then washed successively with aq. HCl (1 M), sat. aq. $NaHCO_3$, and brine, dried over $MgSO_4$ and the solvent was removed under vacuum to give a white solid. Recrystallization from EtOAc gave the *title compound* as a white solid (1.49 g, 75%). Mp 236–238°C. (Found: C 66.15, H 7.22, N 8.04, MH$^+$ 524.2762. $C_{29}H_{37}N_3O_6$ requires C 66.52, H 7.12, N 8.02%, MH$^+$ 524.2760). δ_H ((CD$_3$)$_2$SO) 9.33 (1H, s, C*H*O), 8.05 (1H, d, *J* 8.0, N*H*), 7.55 (1H, d, *J* 7.0, N*H*), 7.30–7.37 (5H, m, Ph), 7.16 (1H, m, N*H*), 7.02 (2H, m, *H*ArO), 6.77 (2H, d, *J* 7.5, *H*ArO), 5.04 (1H, d, *J* 12.5, PhC*H*H), 5.00 (1H, d, *J* 12.5, PhCH*H*), 4.31–4.36 (3H, m, C*H*CH$_2$ArO and ArOC*H*$_2$), 4.18–4.25 (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 4.00–4.07 (2H, m, C*H*CHO and C*H*HCHCHO), 2.86 (1H, dd, *J* 12.0 and 5.0, CHC*H*HArO), 2.63 (1H, m, CHCH*H*ArO), 1.70–1.77 (2H, m, CH*H*CHCHO), 1.46–1.52 (1H, m, CHCH$_2$C*H*(CH$_3$)$_2$), 1.22–1.39 (6H, m, CHC*H*$_2$CH(CH$_3$)$_2$, ArOCH$_2$C*H*$_2$ and CH$_2$C*H*$_2$CHCHO), 0.80–0.83 (6H, m, 2 × CH$_3$). δ_C ((CD$_3$)$_2$SO) 201.8, 172.1, 170.3, 156.6, 156.1, 137.9, 130.1, 129.0, 128.4, 116.2, 66.8, 65.9, 57.2, 56.8, 51.4, 44.1, 31.0, 27.6, 27.1, 24.6, 23.6, 22.2.

(S)-2-[(S)-2-Benzyloxycarbonylamino-3-(4-hydroxyphenyl) propionylamino]-4-methylpentanoic Acid Methyl Ester ***4***

Cbz-L-Tyrosine (5.00 g, 15.9 mmol) and L-leucine methyl ester (2.3 g, 15.9 mmol) were dissolved in DMF (10 mL) under a nitrogen atmosphere. To this solution was added EDCI (3.65 g, 19.0 mmol), HOAt (2.59 g, 19.0 mmol), and DIPEA (6.63 mL, 38.1 mmol). The reaction mixture was stirred overnight at room temperature at which time H_2O and EtOAc were added. The organic phase was separated, washed successively with aq. HCl (1 M, 30 mL), sat. aq. $NaHCO_3$ (300 mL) and brine (300 mL), and then dried over $MgSO_4$. The solvent was removed under vacuum to give the *title compound* as a yellow oil, which was not purified further (5.97 g, 85%). (Found: MH$^+$ 443.2192. $C_{24}H_{31}N_2O_6$ requires 443.2182). δ_H ($CDCl_3$) 7.23–7.32 (5H, m, Ph), 6.94 (2H, app d, *J* 8.5, *H*ArO), 6.81 (1H, d, *J* 7.5, N*H*), 6.68 (2H, app d, *J* 8.5, *H*ArO), 5.70 (1H, d, *J* 8.0, N*H*) 5.03 (2H, s, PhC*H*$_2$), 4.51–4.56 (1H, m, C*H*CH$_2$ArO), 4.42–4.46 (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 3.64 (3H, s, OC*H*$_3$), 2.92–2.99 (2H, m, CHC*H*$_2$ArO), 1.50–1.57 (2H, m, CHCH$_2$C*H*(CH$_3$)$_2$ and CHC*H*HCH(CH$_3$)$_2$), 1.43–1.46 (1H, m, CHCH*H*CH(CH$_3$)$_2$), 0.84 (6H, m, 2 × C*H*$_3$). δ_C ($CDCl_3$) 173.2, 171.8, 156.4, 156.5, 136.3, 130.7, 128.8, 128.5, 128.2, 127.6, 115.9, 67.4, 60.8, 56.5, 52.6, 51.2, 41.5, 37.9, 25.0, 23.1, 22.9, 22.2, 21.4.

(S)-2-[(S)-2-Benzyloxycarbonylamino-3-(4-hydroxyphenyl) propionylamino]-4-methylpentanoic Acid ***5***

Methyl ester **4** (23.00 g, 0.054 mol) was dissolved in MeOH (200 mL) and a solution of LiOH·H$_2$O (11.31 g, 0.270 mol) in H_2O (90 mL) was added. The reaction mixture was stirred overnight at room temperature. The volatiles were removed under vacuum to give a colourless residue that was partitioned between EtOAc and aq. HCl (1 M). The organic layer was separated, washed successively with aq. HCl (1 M) and brine, then dried over $MgSO_4$, and the solvent removed under vacuum to give a glassy solid. Recrystallization from EtOAc gave the *title compound* as a white solid (21.9 g, 96%). Mp 72–74°C. (Found: MH$^+$ 429.2015. $C_{23}H_{29}N_2O_6$ requires 429.2026). δ_H ((CD$_3$)$_2$SO) 9.17 (1H, br s, COO*H*), 8.18 (1H, d, *J* 7.5, N*H*), 7.20–7.37 (5H, m, Ph), 7.07 (2H, m, *H*ArO), 6.63 (2H, m, *H*ArO), 4.93 (2H, s, PhC*H*$_2$), 4.18–4.26 (2H, m, C*H*CH$_2$ArO and C*H*CH$_2$CH(CH$_3$)$_2$), 3.59 (1H, br s, ArO*H*), 2.88 (1H, dd, *J* 14.0 and 3.5, CHC*H*HArO), 2.59 (1H, dd, *J* 14.0 and 11.0, CHCH*H*ArO), 1.61–1.67 (1H, m, CHCH$_2$C*H*(CH$_3$)$_2$), 1.48–1.59 (2H, m, CHC*H*$_2$CH(CH$_3$)$_2$), 0.89 (3H, d, *J* 6.5, C*H*$_3$), 0.85 (3H, d, *J* 6.5, C*H*$_3$). δ_C ($CDCl_3$) 174.8, 172.6, 156.6, 156.5, 137.8, 130.9, 129.0, 128.9, 128.4, 128.1, 115.5, 65.8, 57.0, 50.0, 37.4, 25.0, 23.6, 22.1.

(S)-2-{(S)-2-[(S)-2-Benzyloxycarbonylamino-3-(4-hydroxyphenyl)propionylamino]-4-methylpentanoylamino}-6-hydroxyhexanoic Acid Methyl Ester ***8***

Thionyl chloride (1.2 equiv) was added dropwise to an ice-cooled solution of **6**[7] in methanol (0.01 M). The solution was stirred for 1 h, then allowed to warm to room temperature. The solvent was removed under vacuum to give the hydrochloride salt **7**, which was used without purification.

EDCI (2.70 g, 14.1 mmol), HOAt (1.92 g, 14.1 mmol), and DIPEA (4.90 mL, 28.1 mmol) were added to a solution of **7** (2.30 g, 11.7 mmol) and **5** (5.02 g, 11.7 mmol) in DMF (30 mL) at room temperature. The reaction mixture was stirred under a nitrogen atmosphere for 48 h, and then partitioned between aq.

HCl (1 M) and EtOAc. The organic phase was separated, washed successively with sat. aq. $NaHCO_3$ (200 mL) and brine, dried over $MgSO_4$, and the solvent removed to give the *title compound* as a glassy white solid (5.73 g, 87%). Mp 69–71°C. (Found: MH^+ 572.2984. $C_{30}H_{42}N_3O_8$ requires 572.2972). δ_H (CD_3OD) 7.24–7.37 (5H, m, Ph), 7.03 (2H, app d, *J* 8.5, *H*ArO), 6.67 (2H, app d, *J* 8.5, *H*Ar), 4.97–5.06 (2H, m, OC*H*$_2$Ar), 4.41–4.46 (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 4.32–4.38 (2H, m, C*H*CH$_2$ArO and C*H*CO$_2$Me), 3.69 (3H, s, OC*H*$_3$), 3.50–3.55 (2H, m, C*H*$_2$OH), 2.98–3.03 (1H, m, CHC*H*HAr), 2.73–2.77 (1H, m, CHCH*H*Ar), 1.77–1.87 (1H, m, CHCH$_2$CH(CH$_3$)$_2$), 1.66–1.75 (1H, m, OCH$_2$C*H*HCH$_2$CH$_2$), 1.60–1.64 (1H, m, OCH$_2$CH*H*CH$_2$CH$_2$), 1.51–1.58 (4H, m, CHC*H*$_2$CH(CH$_3$)$_2$ and OCH$_2$CH$_2$C*H*$_2$CH$_2$), 1.38–1.48 (2H, m, OCH$_2$CH$_2$CH$_2$C*H*$_2$), 0.93 (3H, d, *J* 6.5, C*H*$_3$), 0.90 (3H, d, *J* 6.5, C*H*$_3$). δ_C (CD_3OD) 173.5, 173.0, 157.1, 156.1, 137.0, 130.4, 128.4, 128.0, 127.8, 127.5, 115.2, 66.5, 62.4, 61.5, 56.7, 52.7, 40.9, 31.9, 31.1, 24.6, 22.4, 22.1, 21.3.

(S)-2-{(S)-2-[(S)-2-Benzyloxycarbonylamino-3-(4-hydroxyphenyl)propionylamino]-4-methylpentanoylamino}-6-bromohexanoic Acid Methyl Ester ***9***

DDQ (68 mg, 0.3 mmol) was added to a stirred solution of triphenylphosphine (79 mg, 0.3 mmol) in dry DCM (2.5 mL) under a nitrogen atmosphere. TBAB (97 mg, 0.3 mmol) was added and the resulting mustard-brown suspension was stirred for 5 min, at which time **8** (143 mg, 0.25 mmol) was added. The reaction mixture was stirred overnight at room temperature and then diluted with EtOAc. The organic phase was separated, extracted with saturated $NaHCO_3$ until the aqueous layer was no longer yellow, washed with H_2O, dried over $MgSO_4$, and concentrated under vacuum. Recrystallization from EtOAc and pentane gave the *title compound* as a white solid (85 mg, 54%). (Found: MH^+ 634.2139. $C_{30}H_{41}BrN_3O_7$ requires 634.2128). δ_H ($CDCl_3$) 7.39–7.28 (5H, m, Ph), 7.02 (2H, m, *H*ArO), 6.72 (2H, m, *H*ArO), 6.58 (1H, d, *J* 6.5, N*H*), 6.28 (1H, d, *J* 8.5, N*H*), 5.20 (d, *J* 6.5, N*H*), 5.08 (2H, m, OC*H*$_2$Ar), 4.53 (1H, dd, *J* 13.5 and 7.5, C*H*CH$_2$CH(CH$_3$)$_2$), 4.39 (2H, m, C*H*CH$_2$ArO and C*H*CO$_2$Me), 3.75 (3H, s, OC*H*$_3$), 3.35–3.45 (2H, m, C*H*$_2$Br), 3.01 (m, 2H, CHC*H*$_2$Ar), 1.80–1.90 (3H, m, CHCH$_2$C*H*(CH$_3$)$_2$ and BrCH$_2$C*H*$_2$CH$_2$CH$_2$), 1.57–1.75 (2H, m, BrCH$_2$CH$_2$C*H*$_2$CH$_2$), 1.57–1.40 (m, 4H, m, CHC*H*$_2$CH(CH$_3$)$_2$ and BrCH$_2$CH$_2$CH$_2$C*H*$_2$), 0.89 (m, 6H, m, C*H*$_3$).

(S)-2-{(S)-2-[(S)-2-Benzyloxycarbonylamino-3-(4-hydroxyphenyl)propionylamino]-4-methylpentanoylamino}-6-iodohexanoic Acid Methyl Ester ***10***

Method A: To a solution of triphenylphosphine (5.52 g, 0.021 mol) in dry DCM (40 mL) under a nitrogen atmosphere was added DDQ (4.77 g, 0.021 mol). The resulting red solution was treated with TBAI (7.76 g, 0.021 mol) to give a mustard-brown suspension. Stirring was continued for 5 min at which time **8** (8.0 g, 0.014 mol) was added. The reaction mixture was stirred overnight at room temperature, diluted with EtOAc, and the organic phase washed with sat. aq. $NaHSO_3$ until the aqueous layer was no longer yellow in colour. The organic phase was then washed with H_2O, dried over $MgSO_4$ and concentrated under vacuum to give a cream solid. Recrystallization from EtOAc and pentane gave the *title compound* as a white solid (7.15 g, 75%). Mp 108–110°C. (Found: MH^+ 682.1998. $C_{30}H_{41}N_3O_7I$ requires 682.1989). δ_H (CD_3OD) 7.22–7.31 (5H, m, Ph), 7.03 (2H, m, *H*ArO), 6.67 (2H, m, *H*ArO), 4.96–5.05 (2H, m, OC*H*$_2$Ar), 4.43–4.46 (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 4.36–4.38 (2H, m, C*H*CH$_2$ArO and C*H*CO$_2$Me), 3.70 (3H, s, OC*H*$_3$), 3.16–3.19 (2H, m, C*H*$_2$I), 3.00–3.03 (1H, m CHC*H*HAr), 2.73–2.78 (1H, m CHCH*H*Ar), 1.74–1.84 (3H, m, CHCH$_2$C*H*(CH$_3$)$_2$ and ICH$_2$C*H*$_2$CH$_2$CH$_2$), 1.64–1.67 (1H, m, OCH$_2$CH$_2$C*H*HCH$_2$), 1.56–1.63 (3H, m, CHC*H*$_2$CH(CH$_3$)$_2$ and ICH$_2$CH$_2$CH*H*CH$_2$), 1.41–1.48 (2H, m, ICH$_2$CH$_2$CH$_2$C*H*$_2$), 0.90 (3H, d, *J* 6.5, C*H*$_3$), 0.89 (3H, d, *J* 6.5, C*H*$_3$). δ_C (CD_3OD) 173.5, 173.0, 172.6, 157.2, 156.1, 137.0, 130.2, 128.3, 127.9, 127.8, 127.5, 115.1, 66.4, 56.8, 52.3, 51.9, 51.6, 40.8, 37.1, 33.0, 30.2, 26.6, 24.6, 22.3, 21.1.

Method B: To a solution of the alkyl bromide **9** (65 mg, 0.1 mmol) in acetone (1 mL) was added NaI (22 mg, 0.15 mmol). The resulting yellow solution was stirred overnight, concentrated under vacuum, and the residue partitioned between EtOAc and H_2O. The organic phase was separated, washed with H_2O, dried over $MgSO_4$, and concentrated under vacuum to give a yellow solid that had identical data to that reported above.

(7S,10S,13S)-13-Benzyloxycarbonylamino-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic Acid Methyl Ester ***11***

Method A: The iodide **10** (7.0 g, 0.01 mol), K_2CO_3 (2.1 g, 0.015 mol) and Cs_2CO_3 (0.36 g, 1.0 mmol) were dissolved in dry MeCN (30 mL) under a nitrogen atmosphere, and the solution was stirred overnight at reflux. After cooling to room temperature, the solvent was removed under vacuum and the yellow residue partitioned between EtOAc and aq. HCl (1 M). The organic phase was separated, washed successively with aq. HCl (1 M) and brine, dried over $MgSO_4$ and the solvent removed under vacuum to give a yellow solid. This was dissolved in EtOAc, activated charcoal (10 g) was added, and the mixture stirred overnight at room temperature. The solvent was removed under vacuum to give a white solid that was recrystallized from EtOAc and pentane to give the *title compound* as a white solid (3.87 g, 70%). Mp 274–276°C. (Found: C 64.87, H 7.07, N 7.71, MH^+ 554.2859. $C_{30}H_{39}N_3O_7$ requires C 65.08, H 7.10, N 7.59%, MH^+ 554.2866). δ_H (C_5D_5N) 9.44 (1H, d, *J* 9.0, N*H*), 8.88 (1H, d, *J* 8.0, N*H*), 8.52 (1H, d, *J* 8.0, N*H*), 7.33 (2H, m, Ph), 7.18–7.23 (5H, m, Ph and *H*ArO), 6.86 (2H, app d, *J* 8.5, *H*ArO), 5.25 (2H, br s, PhC*H*$_2$), 5.06–5.12 (1H, m, C*H*CH$_2$ArO), 4.83–4.88 (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 3.95 (1H, dd, *J* 14.0 and 7.0, C*H*CO$_2$CH$_3$), 4.22–4.26 (1H, m, ArOC*H*H), 3.41–3.99 (1H, m, ArOCH*H*), 3.54 (3H, s, OCH$_3$), 3.22 (1H, dd, *J* 13.0 and 6.0, CHC*H*HArO), 3.14 (1H, t, m, CHCH*H*ArO, *J* 12.0 and 12.0, CHCH*H*ArO), 1.76–1.82 (4H, m, CHCH$_2$C*H*(CH$_3$)$_2$, CHC*H*HCH(CH$_3$)$_2$, ArOCH$_2$C*H*H and C*H*HCHCO$_2$CH$_3$), 1.60–1.67 (2H, m, C*H*$_2$CHCO$_2$CH$_3$ and CHC*H*$_2$CH(CH$_3$)$_2$), 1.41–1.52 (1H, m, CH$_2$CH*H*CHCO$_2$CH$_3$), 1.21–1.32 (2H, m, ArOCH$_2$CH*H* and CH$_2$CH*H*CHCO$_2$CH$_3$), 0.72 (3H, d, *J* 6.5, CH$_3$), 0.68 (3H, d, *J* 6.5, CH$_3$). δ_C ($CDCl_3$) 172.5, 170.8, 169.7, 157.1, 155.5, 136.3, 130.1, 128.5, 128.2, 128.1, 127.9, 115.7, 66.8, 66.7, 57.1, 52.5, 51.7, 51.2, 43.3, 39.0, 31.5, 28.0, 24.5, 22.9, 22.4, 21.2.

Method B: Pseudo-tripeptide **14** (118 mg, 0.2 mmol) was dissolved in DCM (5 mL) under a nitrogen atmosphere. Trifluoroacetic acid (2 mL) was added and the solution was stirred at room temperature for 3 h. The solvent was removed under vacuum, the residue dissolved in toluene, and the solution re-evaporated to give **15** (76 mg, 0.11 mmol) as

a colourless oil. DMF (5 mL), 2-(7-Aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (46 mg, 0.12 mmol), HOAt (17 mg, 0.12 mmol), and DIPEA (74 μL, 0.44 mmol) were added and the solution was stirred at room temperature overnight before being diluted with ethyl acetate. The organic layer was washed successively with aq. HCl (1 M), sat. aq. $NHCO_3$ and brine, dried over $MgSO_4$ and the solvent removed under vacuum to give the *title compound* as a white solid (27 mg, 31%). Data as above.

*(R)-Methyl 6-(4-((S)-2-(Benzyloxycarbonylamino)-3-((S)-1-*tert*-butoxy-4-methyl-1-oxopentan-2-ylamino)-3-oxopropyl)phenoxy)-2-(*tert*-butoxycarbonylamino) hexanoate* ***14***

Cbz-L-Tyrosine (4.37 g, 13.9 mmol) and L-leucine *tert*-butyl ester (3.10 g, 13.9 mmol) were dissolved in DMF (15 mL) under a nitrogen atmosphere. To this solution was added HATU (5.79 g, 15.2 mmol), HOAt (2.07 g, 15.2 mmol), and DIPEA (9.1 mL, 55.4 mmol). The reaction mixture was stirred at room temperature overnight before H_2O and EtOAc were added. The organic phase was separated, then washed successively with aq. HCl (1 M, 200 mL), sat. aq. $NaHCO_3$ (300 mL), and brine (300 mL) before drying over $MgSO_4$. The solvent was removed under vacuum to give **12** as a white solid (5.39 g, 80%) that was not purified further. Mp 63–65°C. δ_H (300 MHz, $CDCl_3$) 7.27–7.37 (5H, m, Ph), 7.00 (2H, app d, *J* 8.1, *H*ArO), 6.68 (2H, app d, *J* 8.4, *H*ArO), 6.30 (1H, d, *J* 8.1, N*H*), 6.10 (1H, br s, ArO*H*), 5.37 (1H, d, *J* 7.6, N*H*), 5.08 (2H, s, OCH_2Ph), 4.36–4.47 (2H, m, $CHCH_2Ar$ and $CHCH_2CH$), 2.99 (2H, d, *J* 6.4, $CHCH_2Ar$), 1.51–160 (2H, m, $CHCH_2CH(CH_3)_2$), 1.38–1.49 (10H, m, $CHCH_2CH(CH_3)_2$ and $CO_2(CH_3)_3$), 0.89 (6H, app d, *J* 5.8, 2 × CH_3). δ_C (CD_3Cl) 172.0, 171.3, 156.4, 155.6, 136.3, 130.7, 128.8, 128.5, 128.3, 127.6, 115.9, 82.4, 67.4, 56.4, 51.9, 41.9, 37.9, 28.2, 25.1, 22.9, 22.4.

The dipeptide **12** (240 mg, 0.5 mmol), iodide **13**[9] (204 mg, 0.6 mmol), K_2CO_3 (305 mg, 2.2 mmol), and TBAI (61 mg, 0.2 mmol) were dissolved in DMF under a nitrogen atmosphere. The mixture was heated at 60°C for 18 h, allowed to cool to room temperature, and then diluted with EtOAc. The organic phase was washed with H_2O and brine, dried over $MgSO_4$, and the solvent removed under vacuum. The residue was recrystallized from EtOAc to give the *title compound* as a glassy solid (237 mg, 66%). (Found: MH^+ 728.4094. $C_{39}H_{58}N_3O_{10}$ requires 728.4122). δ_H (300 MHz, $CDCl_3$) 7.28–7.37 (5H, m, Ph), 7.02–7.09 (2H, m, *H*ArO), 6.71–6.79 (2H, m, *H*ArO), 6.16 (1H, d, *J* 8.0, N*H*), 5.25–5.30 (1H, m, N*H*), 5.09 (2H, s, OCH_2Ar), 5.03 (1H, d, *J* 7.0, N*H*), 4.29–4.64 (3H, m, $CHCH_2ArO$, $CHCH_2CH(CH_3)_2$ and $CHCO_2CH_3$), 3.88–3.92 (2H, app t, *J* 6.5 and 6.5, $ArOCH_2CH_2$), 3.74 (3H, s, OCH_3), 2.95–3.10 (2H, m, $CHCH_2ArO$), 1.49–1.88 (8H, m, $CHCH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH_2CH_2CH(CH_3)_2$ and $CH_2CH_2CH_2CHCO_2CH_3$), 1.41–1.48 (19H, m, 2 × $(CH_3)_3$ and $CH(CH_3)_2$), 0.88–0.91 (6H, m, 2 × CH_3). δ_C ($CDCl_3$) 173.5, 171.8, 170.9, 170.8, 158.0, 156.0, 155.5, 136.3, 130.5, 128.6, 128.2, 128.0, 115.6, 114.5, 82.0, 80.0, 67.4, 67.0, 56.1, 53.4, 52.3, 51.5, 41.8, 37.6, 32.5, 28.9, 28.4, 28.0, 24.8, 22.8, 22.2.

((7S,10S,13S)-7-Hydroxymethyl-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13-yl)-carbamic Acid Benzyl Ester ***16***

To a suspension of methyl ester **11** (3.5 g, 6.3 mmol) in dry MTBE (20 mL) were added MeOH (0.51 mL, 12.6 mmol) and $LiBH_4$ (0.28 g, 12.6 mmol). The resulting mixture was stirred overnight at room temperature and then washed with aq. HCl (1 M). The organic phase was separated, further washed with H_2O, dried over $MgSO_4$, and the solvent removed under vacuum to give a white solid. Recrystallization from EtOAc gave the *title compound* as a white solid (3.29 g, 99%). Mp 257–259°C. (Found: C 66.11, H 7.02, N 7.54, MH^+ 526.2920. $C_{29}H_{39}N_3O_6$ requires C 66.26, H 7.48, N 7.99%, MH^+ 526.2917). δ_H (CD_3OD) 7.68 (2H, m, Ph), 7.22–7.36 (3H, m, Ph), 7.06 (2H, m, *H*ArO), 6.77 (2H, m, *H*ArO), 5.04 (1H, d, *J* 12.5, PhC*H*H), 4.99 (1H, d, *J* 13.0, PhCH*H*), 4.26–4.34 (2H, m, $CHCH_2ArO$ and ArOC*H*H), 4.06–4.12 (1H, m, ArOCH*H*), 3.96–4.02 (1H, m, $CHCH_2CH(CH_3)_2$), 3.74–3.86 (1H, m, $CHCH_2OH$), 3.30–3.33 (2H, m, CH_2OH), 2.98 (1H, dd, *J* 13.0 and 5.5, CHC*H*HArO), 2.67 (1H, m, CHCH*H*ArO, *J* 12.5 and 12.5, CHCH*H*ArO), 1.75–1.84 (2H, m, CH_2CHCH_2OH), 1.46–1.56 (3H, m, $CHCH_2CH(CH_3)_2$ and $CHCH_2CH(CH_3)_2$), 1.22–1.44 (4H, m, $ArOCH_2CH_2$ and $CH_2CH_2CHCH_2OH$), 0.84 (3H, d, *J* 6.0, CH_3), 0.83 (3H, d, *J* 6.0, CH_3). δ_C (C_5D_5N) 170.6, 169.6, 156.3, 155.5, 137.3, 130.2, 129.3, 128.4, 127.8, 115.6, 109.1, 104.4, 66.6, 65.3, 64.3, 56.3, 50.9, 49.4, 48.1, 43.5, 37.3, 33.5, 30.1, 28.1, 24.0, 23.0, 22.9, 22.2, 20.7.

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