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Novel synthesis of various orthogonally protected C^{α}-methyllysine analogues and biological evaluation of a Vapreotide analogue containing (*S*)-α-methyllysine†

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Prochiral malonic diesters containing a quaternary carbon center have been successfully transformed into a diverse set of ^{t}Boc-Fmoc-$\alpha^{2,2}$-methyllysine-OH analogues through chiral malonic half-ester intermediates obtained *via* enzymatic (Pig Liver Esterase, PLE) hydrolysis. The variety of chiral half-ester intermediates, which vary from 1 to 6 methylene units in the side chain, are achieved in moderate to high optical purity and in good yields. The PLE hydrolysis of malonic diesters with various side chain lengths appears to obey the Jones's PLE model according to the stereochemical configurations of the resulting chiral half-esters. The established synthetic strategy allows the construction of both enantiomers of $\alpha^{2,2}$-methyllysine analogues, and a (*S*)-$\beta^{2,2}$-methyllysine analogue from a common synthon by straightforward manipulation of protecting groups. Two different straightforward and cost effective synthetic strategies are described for the synthesis of $\alpha^{2,2}$-methyllysine analogues. The described strategies should find significant usefulness in preparing novel peptide libraries with unnatural lysine analogues. A Vapreotide analogue incorporating (*S*)-$\alpha^{2,2}$-methyllysine was prepared. However, the Vapreotide analogue with (*S*)-α-methyl-α-lysine is found to lose its specific binding to somatostatin receptor subtype 2 (SSTR2).

Introduction

In recent years there has been considerable interest in the preparation and use of optically pure α,α-disubstituted-α and β nonproteinogenic amino acids in a variety of fields.[1–3] These nonproteinogenic amino acids have been used successfully in the construction of peptides exhibiting unique properties.[4–7] The α,α-disubstituted class of nonproteinogenic amino acids have been used as essential building blocks in the preparation of complex enzyme inhibitors.[3,8,9] The α,α-disubstituted amino acids have demonstrated improved chemical stability, improved hydrophobicity, controlled conformational flexibility of the amino acid side chain, and, hence, constrained conformational freedom of the peptides containing them.[10] The α,α-disubstituted amino acids have been observed to stabilize the secondary structures of peptides by constricting the conformational freedom of the peptide backbone.[11–13] Hence, naturally occurring amino acids have frequently been replaced by α,α-disubstituted non-proteinogenic amino acids in various medicinally important peptides in order to confer more metabolic stability against enzymatic and chemical degradations.[4,6,7,10,13] In addition, the α,α-disubstituted amino acid residues do not undergo *in vivo* racemization, due to the absence of the alpha hydrogen.[14,15] The above examples illustrate the reasons for the growing interest in the use of α,α-disubstituted amino acid analogues.

However, the construction of chiral quaternary carbon centers remains a challenge to the synthetic chemist. Recently Vogt *et al.*[3] reviewed the most widely envisaged synthetic strategies to prepare α,α-disubstituted-α-amino acid analogues that include (i) the asymmetric Strecker reaction starting from aldimines and ketimines or other Strecker related reactions, (ii) electrophilic alkylation of enolates derived from oxazinones, oxazolines, oxazolidines, α-acidamido-β-keto, amino acid derived imines as chiral auxiliaries, (iii) electrophilic-α-amination of α-substituted carbonyl compounds, and (iv) stereospecific ring opening of aziridines, epoxides and rearrangement reactions. Green *et al.*[16] reported the synthesis of α,α-disubstituted-α-amino acids by a Mitsunobu approach, beginning with a chiral α,α-disubstituted-α-hydroxy ester with known stereochemistry. Hartmann *et al.*[17] reported the synthesis of optically enriched α-methyl phenylglycine through L-proline catalyzed

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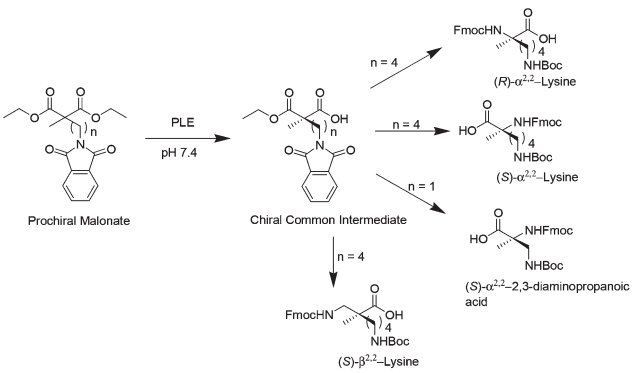
amination of racemic 2-arylpropionaldehydes, using DEAD and DBAD. Cabrera *et al.*[14] established the synthesis of optically pure α,α-disubstituted-α-amino acids employing organocatalyzed Michael addition of racemic oxazolones to α,β-unsaturated aldehydes. Smith *et al.*[18] presented the synthesis of several α,α-disubstituted-α-amino acids from a common intermediate employing nucleophilic "*O*-alkyl fission" ring opening of the NBn_2-α-methylserine lactone, using various organocuprates.

Although there has been extensive research on α,α-disubstituted amino acids, there are very few reports on the preparation of α,α-disubstituted lysine analogues.[15,19–21] In 1988, Seebach *et al.*[20] reported the synthesis of α-methyl-α-lysine in the free, unprotected, form employing the self-regeneration of stereocenters (SRS) principle. However, Seebach's strategy allows synthesizing only α-methyl-α-lysine in moderate yield. Recently, Cativiela *et al.* reported the synthesis of (*S*)-α-methyl-α-lysine *via* chiral cyanopropanoate using a chiral auxiliary in ten steps, but this strategy also allows synthesizing only (*S*)-α-methyl-α-lysine in the free form.[19] To the best of our knowledge Chauhan is the first to report ^{t}Boc-Fmoc protected (*S*)-α-methyl-α-lysine using William's Oxazinone as a chiral auxiliary in eight steps.[15] However, this approach utilizes an expensive chiral auxiliary resulting in the ability to synthesize a single enantiomer of the α-methyl-α-lysine derivative with 90% final purity.

Herein we report a Pig Liver Esterase (PLE) catalyzed desymmetrization approach toward making both orthogonally protected (*R*)- and (*S*)-α-methyl-α-lysine, orthogonally protected (*S*)-α-methyl-β-lysine, and orthogonally protected (*S*)-α-methyl-2,3-diaminopropanoic acid from optically enriched α,α-dialkylmalonic half-esters. Crude PLE is inexpensive and has a proven track record in hydrolyzing a wide variety of prochiral quaternary malonic diesters to the corresponding optically enriched α,α-disubstituted malonic half-esters.[22–26] Our synthetic strategy is convenient and flexible, allowing for the variation of the side chain of lysine analogues from 1 to 6 methylene units, synthesize both enantiomers of an $\alpha^{2,2}$-methyllysine analogue from the same common synthon, and at the same time homologate the (*S*)-$\alpha^{2,2}$-methyllysine to the corresponding (*S*)-$\beta^{2,2}$-methyllysine. We altered the side chain length of lysine in order to probe the effect of chain length in PLE hydrolysis.[27,28] Scheme 1 illustrates our convergent strategy to synthesize various C^{α}-methyllysine analogues from a common synthon type.

In this article we also report a specific binding study of a Vapreotide analogue incorporating α-methyl-α-lysine against the IMR-32 cell line that is known to over express SSTR2 receptors.[29,30] Somatostatin receptors are well known to be over expressed in a broad range of tumour cells.[31] However, native somatostatin (SST) has a short half life *in vivo* due to its rapid degradation by various peptidases.[7] In recent years a variety of SST analogues have been prepared and studied for their potency.[7,30,32–35] Synthetic SST derivatives, such as Vapreotide, have shorter peptide chains compared to native SST. To the best of our knowledge, Prasad *et al.* were the first to



Scheme 1 General synthetic strategy.

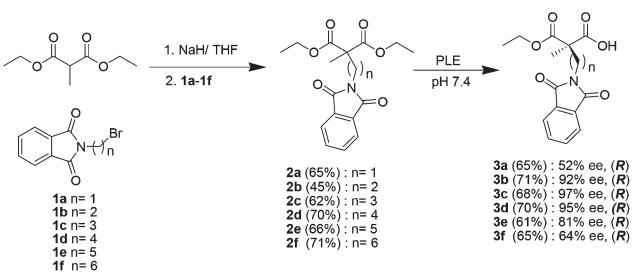
incorporate α,α-disubstituted amino acids to impart improved metabolic stability of SST.[7] We report herein substitution of lysine in Vapreotide with α-methyl-α-lysine, since Trp^{8}–Lys^{9} bond is one of the degradation sites in native SST.[36] Fig. 2 exhibits the Vapreotide analogue which was prepared and studied for specific binding against the IMR32 cell line.

Results and discussion

Synthesis of optically enriched half-ester intermediates

The prochiral malonic diesters (**2a–f**) were synthesized by alkylation of diethyl-2-methylmalonate with the appropriate *N*-(bromoalkyl)-phthalimide as shown in Scheme 2. The resulting diesters, with the exception of **2b**, were purified and isolated in good yield. The poor yield of **2b** was attributed to the dehydrohalogenation of **1b** as evidenced by isolation of significant quantities of alkene. Compounds **2a–2f** were subjected to enzymatic hydrolysis using crude PLE at pH 7.4. The hydrolysis provided enantiomerically enriched half-esters **3a–3f** in good isolated yields as shown in Scheme 2. Surprisingly, PLE was found to provide **3a–f** predominantly of the (*R*)-enantiomer with substantial optical activity in all cases.

Half-esters **3a–3f** have been successfully resolved using chiral HPLC techniques and the enantioselectivity was established by integration of the appropriate chromatographic peaks. The chiral HPLC chromatograms of the half-esters were compared to those of racemic standards of **3a–3f** prepared by standard non-enzymatic methods.



Scheme 2 Preparation of chiral half-esters.

The stereochemical configuration of the major enantiomer of **3a** was determined to have the (*R*) absolute stereochemistry.[37]

The stereochemical configuration of the major enantiomer of **3b** was determined by synthesis[38] as shown in Scheme 8 (ESI†). The optical activity of compound **30** was compared to literature values in order to establish the absolute configuration.

The configurations of **3c** and **3d** were determined by conversion into **31a** and **31b** as shown in Scheme 9 (ESI†). The optical rotations of **31a** and **31b** were compared with the literature values in order to determine the stereochemical configurations of **3c** and **3d**.[20]

The stereochemical configurations of **3e** and **3f** were also determined by synthetic means as shown in Scheme 10 (ESI†).[39–41]

It is evident from Table 1 and Chart 1 that the PLE hydrolysis of diesters **2a–2f** obey the Jones Active Site Model (JASM) (Fig. 1).[28] Diester **2c** provides the highest level of optical purity in the PLE catalyzed hydrolysis reaction. We hypothesize that the size of the side chain is matched with the size of the large hydrophobic pocket in the JASM. The other prochiral diesters having a size mismatch with the large hydrophobic pocket of the JASM results in diminished enantioselectivity with respect to **2c**.

The acid-esters (**3a–f**) were subjected to the Curtius rearrangement resulting in Moz-protected (*S*)-$\alpha^{2,2}$-carbamates (**4a–4f**) in good isolated yields as shown in Scheme 3. Compounds **4a–4f** can be considered as fully protected non-proteinogenic amino acids.

Table 1 % ee of PLE hydrolyzed acid-esters

Diester	$(CH_2)_n$ in the side chain	Half-ester – % ee
2a	1	**3a** – 52%
2b	2	**3b** – 92%
2c	3	**3c** – 97%
2d	4	**3d** – 95%
2e	5	**3e** – 81%
2f	6	**3f** – 64%

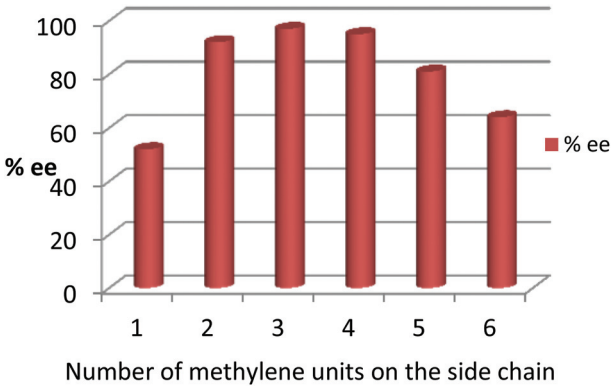


Chart 1 PLE hydrolysis assay of **3a–3f**.

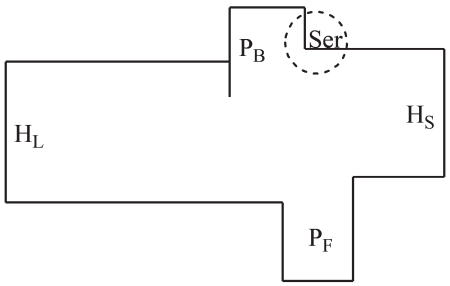
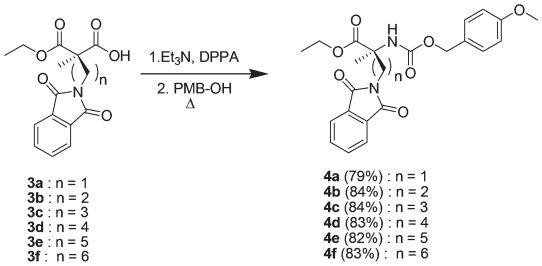


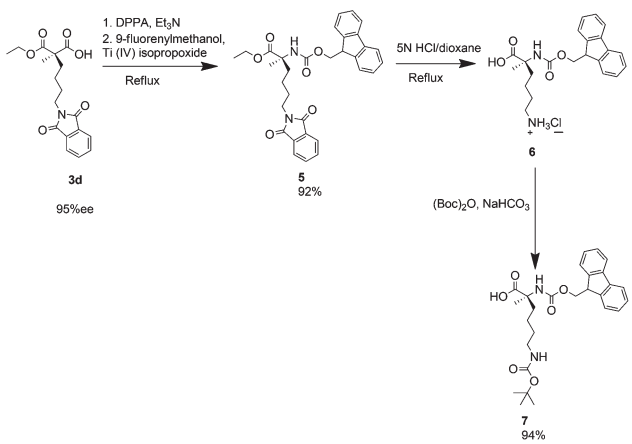
Fig. 1 Jones Active Site Model for Pig Liver Esterase.



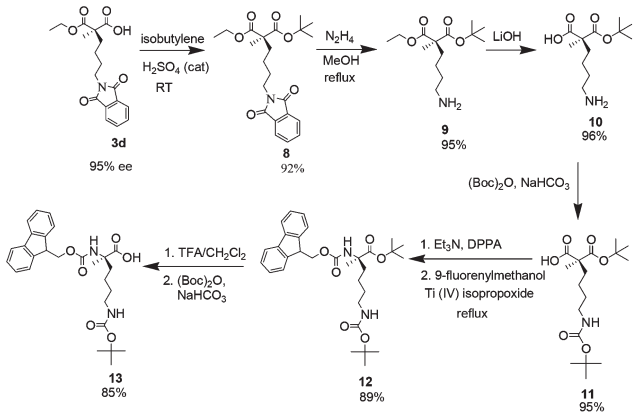
Scheme 3 Conversion of chiral half-esters into protected amino acids.

Synthesis of an orthogonally protected (*S*)-$\alpha^{2,2}$-methyllysine analogue

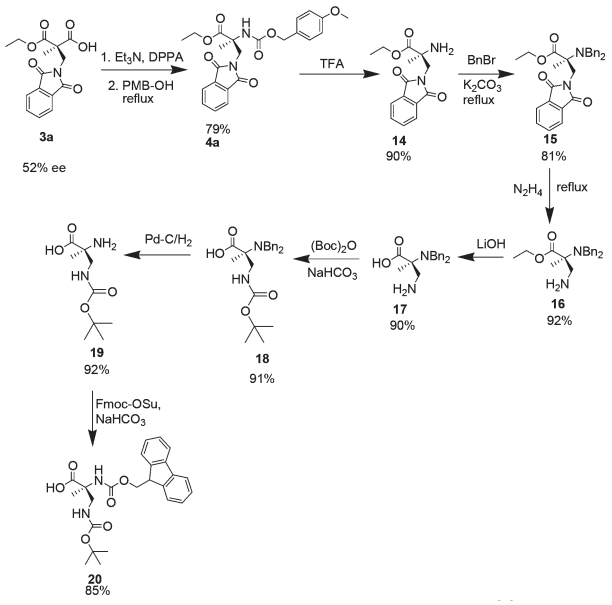
Scheme 4 illustrates the synthesis of ^{t}Boc-Fmoc-(*S*)-$\alpha^{2,2}$-methyllysine-OH (**7**) in three steps starting with the optically enriched synthon **3d**. The bulky quaternary chiral half-ester (**3d**) was directly converted into the Fmoc protected carbamate (**5**) by Ti(IV) isopropoxide promoted Curtius rearrangement with good isolated yield.[42] Acid hydrolysis of the resulting carbamate (**5**) hydrolyzed both the phthalimide and ethyl ester groups leading to the free amino acid (**6**).[43] The obtained **6** was then converted to **7** by reaction with $(Boc)_2O$. Hence, the optimized synthetic strategy is the shortest way to synthesize ^{t}Boc-Fmoc-(*S*)-$\alpha^{2,2}$-methyllysine-OH in overall five steps with good overall yield (42%).



Scheme 4 Synthesis of ^{t}Boc-Fmoc-(*S*)-$\alpha^{2,2}$-methyllysine-OH.



Scheme 5 Synthesis of ^{t}Boc-Fmoc-(*R*)-$\alpha^{2,2}$-methyllysine-OH.



Scheme 6 Synthesis of ^{t}Boc-Fmoc-(*S*)-$\alpha^{2,2}$-2,3-diamino propanoic acid.

Preparation of orthogonally protected (*R*)-$\alpha^{2,2}$-methyllysine analogue

Scheme 5 illustrates the synthesis of (*R*)-$\alpha^{2,2}$-methyllysine analogue **13** in six steps starting from **3d** allowing for an enantiodivergent synthesis of lysine analogues. In order to accomplish the synthesis of **13**, the chiral half-ester **3d** was converted to the mixed diester **8**.[22,24] Compound **8** was subjected to selective deprotection of the phthalimide group resulting in **9**. Compound **9** was saponified producing **10** in excellent yield. Amino acid **10** was converted into **11** using standard reaction conditions. The sterically hindered carboxylic acid **11** was then converted into Fmoc protected α-amino ester **12** in good yield with the implication of Curtius rearrangement using diphenylphosphoryl azide (DPPA) and 9-fluorenylmethanol in presence of catalytic Ti(IV) isopropoxide.[42] However, the chemoselective deprotection of the *tert*-butyl ester in the presence of Boc did not proceed as desired in our hands using known literature procedures.[44–46] We believe this failure is due to the inaccessibility of the sterically hindered ester by those reagents. Hence, we had to treat the amino ester **12** with TFA to deprotect both the *tert*-butyl and Boc groups followed by treatment with $(Boc)_2O$. However this deprotection and reprotection was a one pot strategy that led to the (*R*)-$\alpha^{2,2}$-methyllysine analogue **13** in 8 steps in reasonable overall yield (30%).

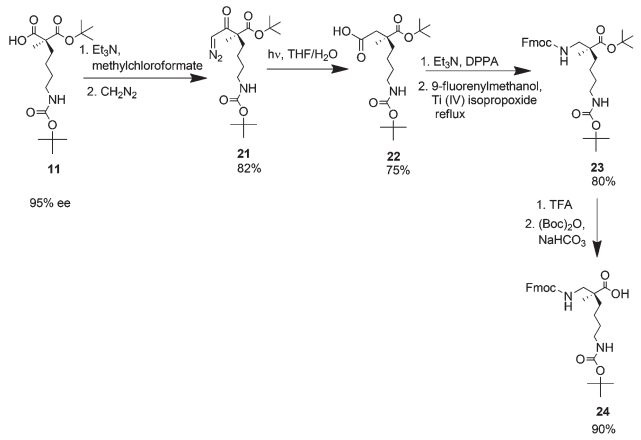
Synthesis of orthogonally protected (*S*)-$\alpha^{2,2}$-2,3-diaminopropanoic acid

Scheme 6 illustrates the synthesis of ^{t}Boc-Fmoc protected (*S*)-$\alpha^{2,2}$-2,3-diaminopropanoic acid (**20**) in eight steps starting with optically enriched half-ester **3a** in good isolated yield. In the first step the chiral half-ester (**3a**) was subjected to a Curtius rearrangement producing the Moz protected α-amino ester (**4a**). The carbamate (**4a**) was then treated with TFA to chemoselectively deprotect the Moz group leading to **14**.[23] Compound **14** was then subject to dibenzylation using excess benzyl bromide (BnBr) and K_2CO_3 at solvent reflux for 48 hours providing **15**.[18] Simple base hydrolysis could not successfully drive the deprotection of the phthalimide group along with ester saponification in a single step starting with the α-dibenzylated aminoester (**15**). This failure is believed to be due to the close proximity of phthalimido group to the bulky quaternary center. However, the chemoselective deprotection of the phthalimide of the dibenzylated amino ester (**15**) using hydrazine resolved the problem providing **16**. Saponification of **16** provides the desired **17**. The free amino acid (**17**) was then selectively protected with the BOC group using Boc anhydride and $NaHCO_3$ in H_2O–dioxane system producing **18** in good isolated yield. The chemoselective hydrogenolysis of **18** led to the α-free amino acid **19** in nearly quantitative yield. The free α-amino acid (**19**) was reprotected with the Fmoc group leading to ^{t}Boc, Fmoc protected amino acid analogue (**20**) in total 10 steps in 14% overall yield.

To the best of our knowledge, Nadir *et al.*[21] is the first group to report the 2,3-diaminopropanoic acid in the free form (unprotected form of **20**), which is inconvenient in terms of solid phase peptide synthesis (SPPS). This success let us synthesize (*S*)-2,3-diaminopropanoic acid suitable for SPPS in eight steps starting with optically enriched chiral half ester **3a**. Additionally, we have recently reported that the optical purity of the acid-ester (**3a**) could be further improved to 95% ee by substituting the crude PLE with PLE Isoenzyme 1, and 2% EtOH as a co-solvent in the biocatalytic hydrolysis of **2a**.[37] Hence, this optimized synthetic strategy is able to provide access to (*S*)-2,3-diaminopropanoic acid in high optical purity, and in properly protected form for SPPS.

Synthesis of (*S*)-Fmoc-$\beta^{2,2}$-methyllysine-Boc-OH

Scheme 7 illustrates the synthesis of orthogonally protected (*S*)-$\beta^{2,2}$-methyllysine (**24**) in eight steps. The chiral half-ester (**11**), that was obtained from **3d** following Scheme 5, was converted into diazoketone (**21**) using standard procedures. The diazoketone (**21**) was subject to photolysis resulting in the γ-keto acid (**22**). The γ-keto acid (**22**) was converted into the



Scheme 7 Synthesis of ^{t}Boc-Fmoc-(*S*)-$\beta^{2,2}$-methyllysine-OH.

Fmoc protected β-amino ester (**23**). β-Amino ester **23** was ultimately converted into the ^{t}Boc-Fmoc-(*S*)-$\beta^{2,2}$-methyllysine (**24**) using well established procedures.

Specific binding study of Vapreotide analogue (25)

Vapreotide® is a widely studied somatostatin analogue with anti-neoplastic properties. Vapreotide has a higher binding affinity to somatostatin receptor subtype 2 (SSTR2) than native somatostatin.[47,48] However, Vapreotide is prone to degradation at the Lys–Val bond by serine proteases (Trypsin, Plasmin, Plasma Kallikrein, *etc.*).[36,49] To the best of our knowledge, Rajeswaran *et al.* is the first to report that the introduction of *N*-methyl-lysine in somatostatin analogues is tolerated and retains the binding affinity to SSTR2.[50] Hence, We have made an effort to prepare a Vapreotide analogue **25** (Fig. 2) replacing naturally occurring lysine with our (*S*)-$\alpha^{2,2}$-methyllysine (**7**) analogue in order to study the specific binding of **25** to SSTR2. The Vapreotide analogue was synthesized using properly protected (*S*)-$\alpha^{2,2}$-methyllysine analogue (**7**). The Vapreotide analogue (**25**) was 99% pure as determined by HPLC.

Specific binding studies of the Vapreotide analogue were conducted against the IMR 32 human neuroblastoma cells. However, it was observed that the Vapreotide analogue showed no specific binding (Table 2 in ESI†) to SSTR2. Hence, a simple switch from naturally occurring lysine to $C^{\alpha,\alpha}$-disubstituted lysine diminishes the specific binding of the Vapreotide analogue (**25**) to SSTR2. We suspect that the loss of specific binding for SSTR2 is attributed to conformational changes of the **25** ring resulting from the introduction of conformationally constrained $C^{\alpha,\alpha}$-disubstituted lysine.[50,51]

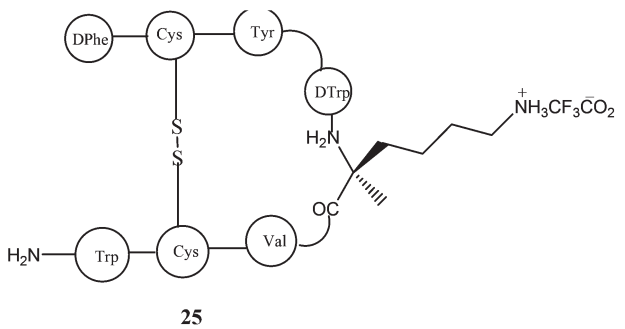


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Fig. 2 Vapreotide® analogue.

Conclusions

We have established two convenient straightforward synthetic strategies to prepare a variety of orthogonally protected $\alpha^{2,2}$-, and $\beta^{2,2}$-methyllysine analogues mediated through inexpensive PLE hydrolysis derived acid-ester intermediates. This optimized technique does not require expensive chiral auxiliaries and reagents to generate the needed chiral quaternary carbon center. In addition, this enantiodivergent methodology allows to construct both D and L-isomers of the orthogonally protected $\alpha^{2,2}$-lysine-OH starting with the enantiomerically enriched common synthon by simple manipulation of the protecting groups. To the best of our knowledge, this is the first time such diverse lysine analogues were synthesized in properly protected form through a common and simple synthetic strategy. Additionally, Scheme 10 (ESI†) provides access to the previously difficult to synthesize α,α-disubstituted amino acids containing hydrophobic side chain in moderate to high % ee *via* a straightforward deamination procedure. The novel α-methyl-α-lysine was incorporated into Vapreotide® to test the effect of the non-proteinogenic lysine on the specific binding of the analogue toward SSTR2 receptors. However, this simple switch from lysine to α-methyllysine results in the loss of specific binding to the SSTR2. Nevertheless, this synthetic strategy should find its usefulness in constructing peptide libraries containing these novel lysine analogues.

Experimental

General methods

THF, CH_2Cl_2, and DMF were dried by passage through a column of activated alumina. All reagents were used as received from commercial sources unless otherwise stated. Melting points were determined in open capillary tubes and are uncorrected. P-60 silica gel was used to conduct flash chromatography. Silica pre-coated TLC plates were used to perform TLC analysis. Normal phase pre-coated silica rotors were chosen to perform radial chromatography. HRMS was obtained using ESI/FTICR-MS and low resolution MS were obtained by ESI/ion trap. Pig Liver Esterase (PLE) is the commercially available crude preparation.

General experimental procedure for the synthesis of malonate esters (2a–2f)

A 250 mL round bottom 3-neck flask fitted with a nitrogen inlet, an addition funnel, and a reflux condenser was charged with 1.2 eq. of NaH (60% dispersion in mineral oil), a stirbar, and 100 mL of dry THF. The resulting suspension was cooled to 0 °C in an icebath. A 50 mL solution of diethyl-2-

methylmalonate (1 eq.) in THF was added over 30 min with stirring. The reaction mixture was then allowed to stir for 60 min at room temperature. A 100 mL solution of *N*-(bromoalkyl)-phthalimide (1 eq.) in THF was added over 30 min with stirring. The reaction mixture was then heated to reflux solvent for 12 h. The solution was cooled to RT, diluted with ether (300 mL), washed twice with 1 N HCl, washed with brine and dried over $MgSO_4$. The resulting suspension was then filtered and the solvent was evaporated *in vacuo*. The resulting yellowish liquid was purified by flash chromatography.

Diethyl 2-(*N*-methylphthalimido)-2-methyl malonate (2a)

2a was prepared following the general procedure for the formation of diester (**2a–2f**) with 10 g (57.4 mmol) diethyl-2-methylmalonate. The resulting yellowish liquid was purified by flash chromatography (30 : 70 EtOAc–hexanes), giving the pure product as a white solid (12.5 g, 65%).[37]

Diethyl 2-(*N*-ethylphthalimido)-2-methyl malonate (2b)

2b was prepared following the general procedure for the formation of diester (**2a–2f**) with 10 g (57.4 mmol) diethyl-2-methylmalonate, 14.6 g (57.4 mmol) *N*-(bromoethyl)-phthalimide and 2.74 g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (18 : 82 EtOAc–hexanes), giving the pure product as a white solid (9 g, 45%).[26]

Diethyl 2-(*N*-propylphthalimido)-2-methyl malonate (2c)

2c was prepared following the general procedure for the formation of diester (**2a–2f**) with 10 g (57.4 mmol) diethyl-2-methylmalonate, 15.4 g (57.4 mmol) *N*-(bromopropyl)-phthalimide and 2.74 g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (30 : 70 EtOAc–hexanes), giving the pure product as a colorless liquid (12.8 g, 62%). R_f = 0.36 (30% EtOAc–hexanes). IR (cm^{-1}) = 2981, 1772, 1705, 1614. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.84 (m, 2H), 7.72 (m, 2H), 4.16 (q, 4H, J = 7 Hz), 3.69 (t, 2H, J = 7 Hz), 1.91 (m, 2H), 1.67 (m, 2H), 1.39 (s, 3H), 1.23 (t, 6H, J = 7 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 53.0, 38.0, 32.5, 23.3, 20.0, 14.0. HRMS ($C_{19}H_{23}NO_6Na^+$) calculated = 384.1423, found = 384.1406.

Diethyl 2-(*N*-butylphthalimido)-2-methyl malonate (2d)

2d was prepared following the general procedure for the formation of diester (**2a–2f**) with 10 g (57.4 mmol) diethyl-2-methylmalonate, 16.2 g (57.4 mmol) *N*-(bromobutyl)-phthalimide and 2.74 g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (30 : 70 EtOAc–hexanes), giving the pure product as a white solid (15.2 g, 70%). R_f = 0.40 (30% EtOAc–hexanes). IR (cm^{-1}) = 2950, 1702. MP = 48 °C. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.85 (m, 2H), 7.71 (m, 2H), 4.17 (q, 4H, J = 7 Hz), 3.67 (t, 2H, J = 7 Hz), 1.90 (m, 2H), 1.69 (m, 2H), 1.39 (s, 3H), 1.27 (m, 8H). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 53.0, 38.0, 35.0, 29.0, 22.0, 20.0, 14.0. HRMS ($C_{20}H_{25}NO_6Na^+$) calculated = 398.1574, found = 398.1573.

Diethyl 2-(*N*-pentylphthalimido)-2-methyl malonate (2e)

2e was prepared following the general procedure for the formation of diester (**2a–2f**) with 10 g (57.4 mmol) diethyl-2-methylmalonate, 17 g (57.4 mmol) *N*-(bromopentyl)-phthalimide and 2.74 g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (30 : 70 EtOAc–hexanes), giving the pure product as a colorless liquid (14.8 g, 66%). R_f = 0.42 (30% EtOAc–hexanes). IR (cm^{-1}) = 2938, 1772, 1706. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.83 (m, 2H), 7.71 (m, 2H), 4.17 (q, 4H, J = 7 Hz), 3.68 (t, 2H, J = 7 Hz), 1.82 (m, 2H), 1.67 (m, 2H), 1.30 (m, 13H). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 54.0, 38.2, 35.2, 28.5, 27.0, 24.0, 20.0, 14.0. HRMS ($C_{21}H_{27}NO_6Na^+$) calculated = 412.1730, found = 412.1728.

Diethyl 2-(*N*-hexylphthalimido)-2-methyl malonate (2f)

2f was prepared following the general procedure for the formation of diester (**2a–2f**) with 10 g (57.4 mmol) diethyl-2-methylmalonate, 17.8 g (57.4 mmol) *N*-(bromopentyl)-phthalimide and 2.74 g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (40 : 60 Et_2O–hexanes), giving the pure product as a colorless liquid (16.5 g, 71%). R_f = 0.44 (30% EtOAc–hexanes). IR (cm^{-1}) = 2940, 1702. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.83 (m, 2H), 7.71 (m, 2H), 4.16 (m, 4H), 3.67 (m, 2H), 1.84 (m, 2H), 1.66 (m, 2H), 1.36 (m, 7H), 1.24 (m, 8H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 54.0, 38.0, 35.0, 29.0, 28.0, 27.0, 24.0, 20.0, 14.0. HRMS ($C_{21}H_{27}NO_6Na^+$) calculated = 426.1893, found = 426.1894.

General experimental procedure for the formation of chiral half-esters (3a–3f)

10 g (1 eq.) of the appropriate malonate (**2a–2f**) was dispersed in 1000 mL of rapidly stirring phosphate buffer (0.1 N, pH 7.4) containing 2% (vol/vol) EtOH as a cosolvent. The pH was maintained using an autotitrator set to maintain a pH of 7.4 and titrate to a volume of 1 eq. NaOH (1.06 M). PLE (27 units per mg, 90 units per mmol of the substrate) was added and the titration was started. The hydrolysis proceeded for 1–6 days depending on substrate. The reaction was stopped when 1 eq. of NaOH was added. The reaction mixture was extracted 3 times with 500 mL of Et_2O. The aqueous layer was then acidified to pH = 1 using 12 M HCl, extracted 8 times with Et_2O. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*.

(*R*)-2-(*N*-Methylphthalimido)-3-ethoxy-2-methyl-3-oxopropanoic acid (3a)

3a was prepared following the general procedure for the formation of half-esters (**3a–3f**) with 10 g (30 mmol) of **2a**. An amount of 6 g (65%) of **3a** was obtained as a white solid in 52% ee.‡[37]

‡The optical purity of **3a** could be greatly improved to 95% using PLE isoenzyme 1, available from Enzymicals, in the biocatalytic asymmetric hydrolysis of **2a**.[37]

(*R*)-2-(*N*-Ethylphthalimido)-3-ethoxy-2-methyl-3-oxopropanoic acid (3b)

3b was prepared following the general procedure for the formation of half-esters (**3a–3f**) with 10 g (29 mmol) of **2b**. An amount of 7.2 g (71%) of **3b** was obtained as a white solid in 92% ee.[26]

(*R*)-2-(*N*-Propylphthalimido)-3-ethoxy-2-methyl-3-oxopropanoic acid (3c)

3c was prepared following the general procedure for the formation of half-esters (**3a–3f**) with 10 g (28 mmol) of **2c**. The resulting half-ester was purified by flash chromatography (40 : 60 EtOAc–hexanes) giving the product as a colorless liquid (6.4 g, 68%). The % ee was determined to be 97% by chiral HPLC (Diacel Chiralpak OJ-H, 4% iPrOH–hexanes, flow rate = 1 mL min^{-1}, λ = 305 nm) $R_{t(S)}$ = 54.9 min (area = 130.13), $R_{t(R)}$ = 58.8 min (area = 7770.41). R_f = 0.22 (40% EtOAc–hexanes). IR (cm^{-1}) = 2983, 2937, 1773, 1747, 1697. $[\alpha]_D^{24}$ = +5.8 (*c* = 2, MeOH). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.85 (m, 2H), 7.73 (m, 2H), 4.21 (q, 2H, *J* = 7 Hz), 3.71 (t, 2H, *J* = 7 Hz), 1.93 (m, 2H), 1.71 (m, 2H), 1.45 (s, 3H), 1.26 (t, 3H, *J* = 7 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 176.0, 172.0, 168.0, 134.0, 132.0, 123.0, 62.0, 53.0, 38.0, 33.0, 24.0, 20.0, 14.0. HRMS ($C_{16}H_{17}NO_6Na^+$) calculated = 356.3256, found = 356.3253.

(*R*)-2-(*N*-Butylphthalimido)-3-ethoxy-2-methyl-3-oxopropanoic acid (3d)

3d was prepared following the general procedure for the formation of half-esters (**3a–3f**) with 10 g (27 mmol) of **2d**, The resulting half-ester was purified by flash chromatography (40 : 60 EtOAc–hexanes) giving the pure product as a white solid (6.6 g, 70%). The % ee was determined to be 95% by chiral HPLC (Diacel Chiralpak OJ-H, 4% iPrOH–hexanes, flow rate = 1 mL min^{-1}, λ = 305 nm) $R_{t(S)}$ = 63.0 min (area = 325.24), $R_{t(R)}$ = 49.6 min (area = 11 659.65). R_f = 0.24 (40% EtOAc–hexane). IR (cm^{-1}) = 3250, 2943, 1718, 1696. MP = 63 °C. $[\alpha]_D^{23}$ = +3.3 (*c* = 1, CH_2Cl_2), ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.82 (bs, 1H), 7.82 (m, 2H), 7.73 (m, 2H), 4.21 (q, 2H, *J* = 7 Hz), 3.71 (t, 2H, *J* = 7 Hz), 1.93 (m, 2H), 1.69 (m, 2H), 1.45 (s, 3H), 1.35 (m, 2H), 1.26 (t, 3H, *J* = 7 Hz), ^{13}C-NMR (CD_3Cl_3, 100 MHz): δ 176.0, 174.0, 169.2, 135.3, 133.2, 124.0, 62.0, 54.3, 39.0, 36.0, 30.0, 23.0, 20.0, 14.0. HRMS ($C_{16}H_{17}NO_6Na^+$) calculated = 370.1261, found = 370.1256.

(*R*)-2-(*N*-Pentylphthalimido)-3-ethoxy-2-methyl-3-oxopropanoic acid (3e)

3e was prepared following the general procedure for the formation of half-esters (**3a–3f**) with 10 g (26 mmol) of **2e**. The resulting half-ester was purified by flash chromatography (40 : 60 EtOAc–hexanes) giving the pure product as a colorless liquid (5.8 g, 61%). The % ee was determined to be 81% by chiral HPLC (Diacel Chiralpak AD-H, 3% iPrOH–hexanes, flow rate = 1 mL min^{-1}, λ = 305 nm) $R_{t(S)}$ = 114.60 min (area = 1591.84), $R_{t(R)}$ = 78.75 min (area = 15 497.54). R_f = 0.28 (40% EtOAc–hexanes). IR (cm^{-1}) = 3250, 2938, 1770, 1700. $[\alpha]_D^{24}$ = +3.2 (*c* = 2, $CHCl_3$), ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.83 (m, 2H), 7.72 (m, 2H), 4.21 (q, 2H, *J* = 7.19), 3.68 (t, 2H, *J* = 7.117), 1.86 (m, 2H), 1.69 (m, 2H), 1.44 (s, 3H), 1.34 (m, 4H), 1.27 (t, 3H, *J* = 7.15), ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 178.0, 172.0, 168.4, 134.0, 132.0, 123.0, 61.4, 54.0, 38.0, 35.5, 28.3, 27.2, 24.0, 20.0, 14.0. HRMS ($C1_9H_{23}NO_6Na^+$) calculated = 384.1417, found = 384.1413.

(*R*)-2-(*N*-Hexylphthalimido)-3-ethoxy-2-methyl-3-oxopropanoic acid (3f)

3f was prepared following the general procedure for the formation of half-esters (**3a–3f**) with 10 g (25 mmol) of **2f**. The resulting half-ester was purified by flash chromatography (40 : 60 EtOAc–hexanes) giving the pure product as a colorless liquid (6.12 g, 61%). The % ee was determined to be 64% by chiral HPLC (Diacel Chiralpak OJ-H, 3% iPrOH–hexanes, flow rate = 1 mL min^{-1}, λ = 305 nm) $R_{t(R)}$ = 57.7 min (area = 13 605 958), $R_{t(S)}$ = 71.7 min (area = 2 954 776). $[\alpha]_D^{24}$ = +2.05 (*c* = 2, $CHCl_3$), ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.83 (m, 2H), 7.72 (m, 2H), 4.21 (q, 2H, *J* = 7.19), 3.68 (t, 2H, *J* = 7.12), 1.86 (m, 2H), 1.69 (m, 2H), 1.44 (s, 3H), 1.34 (m, 4H), 1.27 (t, 3H, *J* = 7.15), ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 177.0, 173.0, 168.4, 134.0, 132.0, 123.0, 61.3, 53.0, 38.0, 35.5, 29.2, 28.2, 26.3, 24.0, 20.0, 14.0. HRMS ($C_{20}H_{25}NO_6Na^+$) calculated = 398.1574, found = 398.1572.

General experimental procedure for the formation of carbamates (4a–4f)

An amount of 10 g (1 eq.) of the appropriate chiral half-ester (**3a–3f**) was dissolved in 50 mL dichloroethane in a 500 mL round bottom flask with a stirbar under a N_2 atmosphere. A measured volume of Et_3N (2.1 eq.) and diphenylphosphorylazide (DPPA) (1.1 eq.) was added to the solution and the solution was allowed to stir at RT for 90 min. At this point the reaction was heated to reflux solvent for 2 h. A measured volume of *para*-methoxybenzyl alcohol (PMB-OH) (1.4 eq.) was added to the reaction mixture and the reaction was continued to reflux solvent for 12 h. The reaction was cooled and diluted with CH_2Cl_2, filtered through a silica bed (1″ bed in a Buchner funnel) and evaporated. The resulting residue was purified by flash chromatography (40% EtOAC–hexanes) giving the pure product as a white wax or colorless viscous oil.

4-Methoxybenzyl-(*S*)-2-(ethoxycarbonyl)-1-(1,3-dioxoisoindolin-2-yl)propan-2-ylcarbamate (4a)

4a was prepared following the general synthetic procedure for the formation of carbamates (**4a–4f**). An amount of 11.7 g (26.5 mmol, 79%) of product was obtained as a white wax. R_f = 0.25 (40% EtOAc–hexanes). IR (cm^{-1}) = 3368, 2958, 1774, 1708, 1612. $[\alpha]_D^{23}$ = −1.2 (*c* = 1.2, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.84 (m, 2H), 7.73 (bs, 2H), 7.31 (d, 2H, *J* = 9 Hz), 6.87 (d, 2H, 9 Hz), 6 (bs, 1H), 5 (q, 2H, *J* = 12 Hz), 4.18 (m, 2H), 4.12 (s, 2H), 3.8 (s, 3H), 1.65 (S, 3H), 1.25 (m, 3H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 171.0, 168.5, 159.0, 155.0, 134.0, 132.0, 130.0, 128.4, 123.0, 113.3, 66.0, 62.0, 60.2, 55.0, 43.4, 20.0, 14.0. HRMS ($C_{23}H_{24}N_2O_7$) calculated = 463.1476, found = 463.1469.

4-Methoxybenzyl-(*S*)-2-(ethoxycarbonyl)-4-(1,3-dioxoisoindolin-2-yl)butan-2-ylcarbamate (4b)

4b was prepared following the general synthetic procedure for the formation of carbamates (**4a–4f**). An amount of 12 g (26.4 mmol, 84%) of product was obtained as a colorless viscous oil. R_f = 0.29 (35% EtOAc–hexanes). IR (cm^{-1}) = 3353, 2952, 1771, 1704, 1612. $[\alpha]_D^{23}$ = +11.3 (c = 1, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.81 (m, 2H), 7.69 (m, 2H), 7.28 (m, 2H), 6.87 (m, 2H), 5.78 (bs, 1H), 4.94 (m, 2H), 4.07 (m, 2H), 3.80 (s, 3H), 3.67 (m, 2H), 2.57 (bm, 1H), 2.36 (m, 1H), 1.60 (s, 3H), 1.12 (t, 3H, J = 7 Hz), ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 173.0, 168.0, 159.0, 154.0, 134.0, 132.0, 130.0, 128.6, 123.0, 114.0, 66.0, 62.0, 58.0, 55.0, 34.0, 33.6, 24.0, 14.0. HRMS ($C_{24}H_{26}N_2O_7Na^+$) calculated = 477.1638, found = 477.1635.

4-Methoxybenzyl-(*S*)-2-(ethoxycarbonyl)-5-(1,3-dioxoisoindolin-2-yl)pentan-2-ylcarbamate (4c)

4c was prepared following the general synthetic procedure for the formation of carbamates (**4a–4f**). An amount of 11.8 g (25.2 mmol, 84%) of product was obtained as a colorless viscous oil. R_f = 0.27 (35% EtOAc–hexane). IR (cm^{-1}) = 3359, 2939, 1770, 1702, 1612. $[\alpha]_D^{23}$ = −6.0 (c = 0.8, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.82 (m, 2H), 7.70 (m, 2H), 7.28 (m, 2H), 6.88 (m, 2H), 5.63 (bs, 1H), 4.95 (s, 2H), 4.16 (m, 2H), 3.79 (s, 3H), 3.65 (m, 2H), 2.26 (m, 1H), 1.84 (m, 1H), 1.69 (m, 1H), 1.54 (s, 3H), 1.48 (m, 1H), 1.21 (t, 3H, J = 7 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 174, 168, 160, 154, 134, 132, 130, 128, 123, 114, 66, 62, 60, 55, 38, 34, 23.5, 23.4, 14. HRMS ($C_{25}H_{28}N_2O_7Na^+$) calculated = 491.1789, found = 491.1782.

4-Methoxybenzyl-(*S*)-2-(ethoxycarbonyl)-6-(1,3-dioxoisoindolin-2-yl)hexan-2-ylcarbamate (4d)

4d was prepared following the general synthetic procedure for the formation of carbamates (**4a–4f**). An amount of 11.5 g (24 mmol, 83%) of product was obtained as a colorless viscous oil. R_f = 0.31 (35% EtOAc–hexanes). IR (cm^{-1}) = 3360, 2958, 1768, 1701, 1612. $[\alpha]_D^{23}$ = −1.5 (c = 1.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.82 (m, 2H), 7.69 (m, 2H), 7.30 (m, 2H), 6.88 (m, 2H), 5.62 (bs, 1H), 4.99 (s, 2H), 4.17 (m, 2H), 3.8 (s, 3H), 3.63 (t, 2H, J = 7 Hz), 2.17 (m, 1H), 1.83 (m, 1H), 1.65 (m, 2H), 1.56 (s, 3H), 1.33 (m, 1H), 1.23 (t, 3H, J = 7 Hz), 1.12 (m, 1H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 174.0, 168.0, 159.5, 154.6, 134.0, 132.1, 130.0, 128.7, 123.2, 114.0, 66.2, 62.0, 60.0, 55.3, 38.0, 36.0, 28.4, 23.4, 21.4, 14.1. HRMS ($C_{26}H_{30}N_2O_7Na^+$) calculated = 505.1945, found = 505.1930.

4-Methoxybenzyl-(*S*)-2-(ethoxycarbonyl)-7-(1,3-dioxoisoindolin-2-yl)heptan-2-ylcarbamate (4e)

4e was prepared following the general synthetic procedure for the formation of carbamates (**4a–4f**). An amount of 11.5 g (23 mmol, 82%) of product was obtained as a colorless viscous oil. R_f = 0.33 (35% EtOAc–hexanes), IR (cm^{-1}) = 3367, 2938, 1770, 1703, 1612. $[\alpha]_D^{23}$ = +1.4 (c = 1, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.83 (m, 2H), 7.70 (m, 2H), 7.28 (d, 2H, J = 9 Hz), 6.87 (m, 2H, J = 9 Hz), 5.63 (bs, 1H), 4.99 (s, 2H), 4.18 (m, 2H), 3.79 (s, 3H), 3.64 (t, 2H, J = 7 Hz), 2.12 (bm, 1H), 1.76 (m, 1H), 1.64 (m, 2H), 1.55 (s, 3H), 1.27 (m, 7H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 174.0, 168.0, 159.0, 155.0, 134.0, 132.0, 130.0, 129.0, 123.0, 114.0, 66.0, 61.0, 60.0, 55.0, 38.0, 36.0, 28.0, 27.0, 24.0, 23.5, 14.0. HRMS ($C_{27}H_{32}N_2O_7Na^+$) calculated = 519.2102, found = 519.2095.

4-Methoxybenzyl-(*S*)-2-(ethoxycarbonyl)-8-(1,3-dioxoisoindolin-2-yl)octan-2-ylcarbamate (4f)

4f was prepared following the general synthetic procedure for the formation of carbamates (**4a–4f**). An amount of 11.3 g (22 mmol, 83%) of product was obtained as colorless viscous oil. R_f = 0.34 (35% EtOAc–hexanes). IR (cm^{-1}) = 3366, 2936, 2859, 1770, 1703, 1612. $[\alpha]_D^{23}$ = −1.7 (c = 1.4, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.74 (m, 2H), 7.62 (m, 2H), 7.22 (d, 2H, J = 7 Hz), 6.80 (d, 2H, J = 8 Hz), 5.51 (bs, 1H), 4.91 (s, 2H), 4.11 (m, 2H), 3.73 (s, 3H), 3.58 (t, 2H, J = 7 Hz), 2.04 (m, 1H), 1.67 (m, 1H), 1.56 (m, 2H), 1.47 (s, 3H), 1.20 (m, 8H), 0.98 (bm, 1H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 173.0, 167.0, 157.0, 154.0, 133.0, 131.0, 129.0, 128.0, 122.0, 113.0, 65.0, 60.0, 59.0, 54.0, 37.0, 36.0, 28.0, 27.0, 26.0, 23.0, 22.0, 13.0. HRMS ($C_{28}H_{34}N_2O_7Na^+$) calculated = 533.2258, found = 533.2251.

Synthesis of (9*H*-fluoren-9-yl) methyl (*S*)-2-(ethoxycarbonyl)-6-(1,3-dioxoisoindolin-2-yl)hexan-2-ylcarbamate (5)

A volume of 320 μL Et_3N (2.3 mmol) was added to a solution of 0.7 g (1.9 mmol) **3d** in 25 mL dichloroethane under a N_2 atmosphere. A volume of 460 μL DPPA (2 mmol) was added to the reaction mixture. The mixture was allowed to stir at RT for 2 h. The mixture was then heated to reflux solvent for 3 h. The reaction was cooled and washed with saturated NH_4Cl solution. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure giving the crude isocyanate. The isocyanate was dissolved in dry toluene under a N_2 atmosphere. An amount of 0.75 g (3.8 mmol) 9-fluorenylmethanol and a volume of 66 μL Ti(IV) isopropoxide was added to the solution. The mixture was heated to 80 °C for 12 h. The mixture was cooled and the toluene was evaporated under reduced pressure giving the crude product. The residue was purified by chromatography (10% hexanes–CH_2Cl_2) giving 0.95 g **5** (1.75 mmol, 92%) as a white solid. R_f = 0.29 (10% hexanes–CH_2Cl_2). IR (cm^{-1}) = 3365, 2940, 1769, 1704, 1613, 1504. MP = 81 °C, $[\alpha]_D^{22}$ = −14.3 (c = 1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.78 (m, 4H), 7.64 (m, 4H), 7.40 (t, 2H, J = 7 Hz), 7.32 (t, 2H, J = 7 Hz), 5.72 (bs, 1H), 4.35 (bm, 2H), 4.20 (bm, 3H), 3.65 (bm, 2H), 2.21 (bm, 1H), 1.85 (bm, 1H), 1.60 (m, 5H), 1.35 (bm, 1H), 1.24 (bm, 3H), 1.13 (bm, 1H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 174.0, 168.0, 144.0, 141.0, 134.0, 132.0, 128.0, 127.0, 125.0, 123.0, 120.0, 66.0, 62.0, 60.0, 47.0, 37.5, 36.0, 28.0, 23.4, 21.0, 14.0. HRMS ($C_{32}H_{32}N_2O_6Na^+$) calculated = 563.2152, observed = 563.2144.

Fluorenylmethyloxycarbonylamino-2-methylhexanoic acid·HCl (6)

6 was synthesized from **5** following a literature published procedure.[52] An amount of 0.9 g (1.7 mmol) **5** was dissolved in 12 mL 1,4-dioxane. A volume of 12 mL 5 N HCl was added to the solution. The solution was heated to reflux solvent for

24 h. At which time the reaction was found to be completed by ESI-MS (ESI†). The solution was concentrated under reduced pressure and the residue was taken for the next step without further purification.

Synthesis of (*S*)-^{t}Boc-Fmoc-α-methyl-α-lysine-OH (7)

An amount of 0.25 g $NaHCO_3$ (3 mmol) was added to a solution of 0.6 g of **6** (~1.5 mmol) in 15 mL of water with stirring. The solution was cooled to 0 °C. A solution of 0.65 g $(Boc)_2O$ (3 mmol) in 15 mL 1,4-dioxane was added to the reaction mixture over 20 min. The reaction was allowed to stir at 0 °C for an hour and then at ambient temperature for 12 h. The mixture was given pentane wash to remove excess $(Boc)_2O$. The reaction mixture was diluted with 30 mL of water, acidified to pH 4 with 2 M HCl, and extracted (3 × 50 mL) with Et_2O. The combined ether layer was washed with brine, dried over $MgSO_4$, evaporated under reduced pressure giving the crude product as light yellow oil. The residue was purified by radial chromatography using 5% MeOH–CH_2Cl_2 giving 0.78 g (1.6 mmol, 94% over two steps) of product as a white solid. R_f = 0.33 (5% MeOH–CH_2Cl_2). IR (cm^{-1}) = 3350, 2941, 1681, 1504. MP = 95 °C. $[\alpha]_D^{22}$ = +14.4 (*c* = 1, $CHCl_3$), ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.69 (d, 2H, *J* = 8 Hz), 7.55 (d, 2H, *J* = 8 Hz), 7.28 (t, 2H, *J* = 8 Hz), 7.21 (t, 2H, *J* = 8 Hz), 4.22 (d, 2H, *J* = 7 Hz), 4.11 (t, 1H, *J* = 7 Hz), 2.92 (t, 2H, *J* = 7 Hz), 1.77 (bs, 2H), 1.33 (m, 16H). ^{13}C-NMR (CD_3OD, 100 MHz): 176.0, 157.0, 155.0, 144.0, 143.9, 141.0, 127.0, 126.7.0, 125.0, 119.0, 78.0, 66.0, 59.0, 40.0, 36.0, 29.0, 27.0, 21.0, 20.0. HRMS ($C_{27}H_{34}N_2O_6Na^+$) calculated = 505.2309, observed = 505.2296.

Synthesis of (*S*)-1-*tert*-butyl 3-ethyl 2-[4-(1,3-dioxoisoindolin-2-yl)butyl]-2-methylmalonate (8)

A volume of 3 mL conc. H_2SO_4 was added to a solution of 10 g **3d** (29 mmol) in 100 mL CH_2Cl_2 in a 250 mL sealed tube. The solution was cooled to −7 °C in an ice salt bath. A volume of 50 mL of condensed isobutylene was added to the solution. The tube was capped tightly and allowed to stir over night at RT. The tube was uncapped and allowed to stir for 2 h at ambient pressure to allow the excess isobutylene to evaporate. The solution was diluted with CH_2Cl_2 and gently washed three times with 1 N NaOH (50 mL). The CH_2Cl_2 layer was dried over $MgSO_4$, evaporated under reduced pressure, and purified by chromatography (40% EtOAC–hexanes) giving 10.8 g of product (26.7 mmol, 92%) as a colorless liquid. R_f = 0.60 (40% EtOAc–hexanes), IR (cm^{-1}) = 2977, 2937, 1771, 1707. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.84 (m, 2H), 7.71 (m, 2H), 4.16 (q, 2H, *J* = 7 Hz), 3.68 (t, 2H, *J* = 7 Hz), 1.85 (m, 2H), 1.69 (m, 2H), 1.42 (s, 9H), 1.28 (m, 8H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.0, 171.0, 168.0, 134.0, 132.0, 123.0, 81.0, 61.0, 54.0, 38.0, 35.0, 29.0, 28.0, 22.0, 20.0, 14.0. HRMS ($C_{22}H_{29}NO_6Na^+$) calculated = 426.1887, observed = 426.1873.

Synthesis of (*S*)-1-*tert*-butyl 3-ethyl 2-(4-aminobutyl)-2-methylmalonate (9)

A volume of 2.8 mL (31.4 mmol) $N_2H_4{\cdot}H_2O$ (35% in H_2O) was added in a solution of 10.5 g **8** (26 mmol) in 60 mL MeOH. The solution was heated to reflux solvent for 6 h. The reaction mixture was found to turn turbid and a white precipitate formed within 2 h of reflux. The reaction was monitored by TLC. The reaction was cooled to RT and the MeOH was removed *in vacuo*. The residue was taken up in CH_2Cl_2 and the white precipitate was filtered off. The CH_2Cl_2 was evaporated under reduced pressure giving 6.75 g (24.7 mmol, 95%) of **9** as a colorless oil. R_f = 0.16 (5% MeOH–CH_2Cl_2), IR (cm^{-1}) = 3395, 2977, 2934, 2867, 1723, 1654. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 4.17 (q, 2H, *J* = 7 Hz), 2.70 (t, 2H, *J* = 7 Hz), 1.82 (m, 2H), 1.53 (bs, 2H), 1.45 (m, 11H), 1.35 (s, 3H), 1.27 (m, 5H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.0, 171.0, 81.0, 61.0, 54.0, 42.0, 35.0, 34.0, 28.0, 21.0, 19.0, 14.0. HRMS ($C_{14}H_{27}NO_4Na^+$) calculated = 296.1832, observed = 296.1828.

Synthesis of (*S*)-2-(*tert*-butoxycarbonyl)-6-amino-2-methylhexanoic acid (10)

An amount of 1.76 g LiOH (73.5 mmol) was added in a solution of 6.7 g **9** (24.5 mmol) in 30 mL of 3 : 7 EtOH–H_2O mixture. The solution was allowed to stir for 48 h at RT. The solvents were evaporated under reduced pressure upon completion as determined by TLC (5% MeOH–CH_2Cl_2). The residue was triturated with MeOH to precipitate excess LiOH. The MeOH layer was evaporated under reduced pressure giving 5.76 g of **10** (96%, 23.5 mmol) as a white wax. R_f = 0.10 (5% MeOH–CH_2Cl_2). IR (cm^{-1}) = 3297, 2961, 2937, 1541, 1448. ^{1}H-NMR (CD_3OD, 400 MHz): δ 2.64 (t, 2H, *J* = 7 Hz), 1.79 (m, 2H), 1.46 (m, 11H), 1.29 (m, 5H). ^{13}C-NMR (CD_3OD, 100 MHz): δ 178.0, 175.0, 80.0, 56.0, 41.0, 36.0, 33.0, 26.0, 21.0, 20.0. ESI-MS ($C_{12}H_{23}NO_4Na^+$) calculated 268.3, observed 268.2.

Synthesis of (*S*)-2-(*tert*-butoxycarbonyl)-6-(*tert*-butyloxycarbonylamino)-2-methylhexanoic acid (11)

An amount of 3.9 g $NaHCO_3$ (46.5 mmol) was added to a solution of 5.7 g of **10** (23.2 mmol) in 20 mL H_2O. The solution was cooled to 0 °C. A solution of 6 g of $(Boc)_2O$ (28 mmol) in 20 mL 1,4-dioxane was added drop wise to the reaction mixture. The reaction was allowed to stir at 0 °C for an hour and then brought to RT. The reaction was allowed to stir at RT for 12 h. The reaction mixture was extracted with pentane. The aqueous layer was acidified to pH 4 using 2 N HCl and extracted three times with Et_2O (50 mL). The combined ether layer was dried over $MgSO_4$, evaporated under reduced pressure, and purified by chromatography (40% EtOAc–hexanes) giving 7.6 g (22 mmol, 95%) of **11** as a colorless viscous oil. R_f = 0.54 (40% EtOAc–hexanes), IR (cm^{-1}) = 3380, 2976, 2934, 1706, 1522. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 4.61 (bs, 1H), 3.12 (bm, 2H), 1.84 (m, 2H), 1.46 (m, 23H), 1.29 (m, 2H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 177.0, 172.0, 156.0, 82.0, 79.0, 54.0, 40.0, 35.0, 30.0, 28.4, 27.8, 22.0, 20.0. HRMS ($C_{17}H_{31}NO_6Na^+$) calculated = 368.2043, observed = 368.2035.

Synthesis of (*R*)-*tert*-butyl-2-(9-fluorenylmethylamino)-6-(*tert*-butyloxycarbonylamino)-2-methylhexanoate (12)

A volume of 2.7 mL of Et_3N (19.4 mmol) was added to a solution of 5.6 g **11** (16.2 mmol) in 60 mL dichloroethane under a

N_2 atmosphere. A volume of 3.9 mL (17.3 mmol) of DPPA was added to the reaction mixture. The mixture was allowed to stir at RT for 2 h. The mixture was heated to reflux solvent for 3 h. The reaction was cooled and the organic layer was extracted with saturated NH_4Cl solution, dried over $MgSO_4$ and evaporated under reduced pressure giving the isocyanate. The isocyanate was taken up in dry toluene under a N_2 atmosphere. An amount of 6.4 g (32.4 mmol) 9-fluorenylmethanol was added to the solution along with 300 μL of Ti(IV) isopropoxide. The solution was heated to 80 °C over night. The reaction was cooled and the organic layer was evaporated under reduced pressure. The residue was then purified by chromatography (CH_2Cl_2) giving 7.75 g (14.4 mmol, 89%) of **12** as colorless wax. An amount of 50 mg of **12** was further purified by reversed phase HPLC (40% CH_3CN–H_2O to 100% CH_3CN in 15 min at 262 nm, R_t = 16.6 min) giving 35 mg of pure **12** as a colorless oil. R_f = 0.57 (CH_2Cl_2), IR (cm^{-1}) = 3359, 2975, 2931, 1707, 1516. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.76 (d, 2H, J = 8 Hz), 7.61 (d, 2H, J = 8 Hz), 7.40 (t, 2H, J = 7 Hz), 7.32 (t, 2H, J = 7 Hz), 5.82 (bs, 1H), 4.47 (m, 2H), 4.18 (t, 1H, J = 7 Hz), 4.02 (bs, 1H), 3.07 (bm, 2H), 2.23 (bm, 1H), 1.74 (bm, 1H), 1.45 (m, 25H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 173.2, 156.03, 154.3, 143.9, 141.4, 127.6, 127.0, 125.1, 120.0, 82.2, 79.2, 66.3, 60.1, 47.2, 40.1, 35.8, 29.7, 28.3, 27.9, 23.7, 21.2. HRMS ($C_{31}H_{42}N_2O_6Na^+$) calculated = 561.2935, observed = 561.2924.

Synthesis of (*R*)-^{t}Boc-Fmoc-α-methyl-α-lysine-OH (13)

An amount of 2 g (3.7 mmol) of **12** was dissolved in 20 mL of 1 : 1 TFA–CH_2Cl_2. The solution was allowed to stir for 12 h at RT under a N_2 atmosphere. The reaction was monitored by TLC (5% MeOH–CH_2Cl_2) and ESI-mass spectrometry for completion. At which point the TFA–CH_2Cl_2 layer was evaporated under reduced pressure giving free amino acid. The residue was taken up in 15 mL of H_2O and 0.76 g (9 mmol) of $NaHCO_3$ was added to the solution slowly to control the effervescence. The mixture was cooled to 0 °C. A solution of 0.96 g (4.4 mmol) $(Boc)_2O$ in 15 mL 1,4-dioxane was added to the mixture slowly at 0 °C. The reaction was allowed to stir at 0 °C for an hour. The reaction was then allowed to warm to RT and stir for 12 h. The reaction mixture was extracted with pentane to remove excess $(Boc)_2O$. The aqueous layer was then acidified to pH 4 with 2 N HCl, extracted three times with Et_2O (50 mL). The combined ether layer was dried over $MgSO_4$, evaporated under reduced pressure and purified by chromatography (5% MeOH–CH_2Cl_2) giving 1.52 g (3.15 mmol, 85% over two steps) of **13** as a white solid similar to **7**. All characterization data of **13** complied with the data for **7**. The polarimetry reading confirmed **13** as the enantiomer to **7**. $[\alpha]_D^{22}$ = −11.5 (c = 1, $CHCl_3$).

Synthesis of (*S*)-ethyl 2-amino-2-((1,3-dioxoisoindolin-2-yl)-methyl) propanoate (14)

An amount of 10 g (23 mmol) **4a** was dissolved in 60 mL of methylene chloride and 10 mL TFA was added. The solution was stirred for 1 h. The solution became dark purple in color. A volume of 100 mL H_2O was added to the solution and the organic layer was washed with $NaHCO_3$ solution, washed with H_2O, and dried over $MgSO_4$. The residue was purified by flash chromatography (5% MeOH–CH_2Cl_2) giving 5.7 g (20.6 mmol, 90%) of **14** as a white wax. R_f = 0.64 (5% MeOH–CH_2Cl_2), IR (cm^{-1}) = 3391, 3325, 3000, 2959, 1770, 1731, 1704, 1557. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.85 (m, 2H), 7.73 (m, 2H), 4.20 (m, 2H), 3.91 (m, 2H), 1.77 (bs, 2H), 1.41 (s, 3H), 1.29 (t, 3H, J = 7 Hz), ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 175.0, 169.0, 134.0, 132.0, 123.0, 61.0, 58.0, 46.0, 24.0, 14.0. HRMS ($C_{14}H_{16}N_2O_4Na^+$) calculated = 299.1002, observed = 299.1002.

Synthesis of (*S*)-ethyl 2-(dibenzylamino)-2-[(1,3-dioxoisoindolin-2-yl)methyl]propanoate (15)

An amount of 4.2 g (15.2 mmol) of **14** was dissolved in 60 mL of distilled acetonitrile in a 250 mL three necked flask under a N_2 atmosphere. An amount of 12.6 g (91.2 mmol) of K_2CO_3 was added with stirring. A volume of 9 mL (76 mmol) of BnBr was added drop wise. The reaction mixture was heated to reflux solvent for 12 h. The reaction mixture was diluted with 50 mL of H_2O and the solution was extracted with ether three times. The ether layer was washed with H_2O, washed with brine, dried over $MgSO_4$ and evaporated. The residue was then purified by flash chromatography (30% EtOAc–hexanes) giving 5.6 g (12.3 mmol, 81%) of **15** as a white solid. R_f = 0.59 (30% EtOAc–hexanes), IR (cm^{-1}) = 2970, 1770, 1713, 1620. MP = 83 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.83 (m, 2H), 7.71 (m, 2H), 7.34 (d, 4H, J = 8 Hz), 7.12 (m, 6H), 4.23 (q, 2H, J = 7 Hz), 3.95 (m, 6H), 1.35 (m, 6H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 174.0, 168.0, 141.0, 134.0, 132.0, 128.3, 128.0, 126.0, 123.0, 68.0, 61.0, 55.0, 44.0, 19.0, 14.0. HRMS ($C_{28}H_{28}N_2O_4Na^+$) = 479.1941, observed = 479.1938.

(*S*)-Ethyl-3-amino-2-(dibenzylamino)-2-methylpropanoate (16)

An amount of 3.2 g (7 mmol) of **15** was dissolved in 20 mL of (8 : 2) MeOH and CH_2Cl_2. A volume of 1.7 mL (21 mmol) of N_2H_4 (35% in H_2O) was added. The solution was heated to reflux solvent for 3 h. The formation of a white precipitate indicated the completion of the reaction. The reaction mixture was filtered and the filtrate was evaporated giving 2 g (6.5 mmol, 92%) of **16** as a yellowish oil. R_f = 0.65 (5% MeOH–CH_2Cl_2). IR (cm^{-1}) = 2979, 1717, 1601. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.21 (m, 10H), 4.16 (m, 2H), 3.84 (m, 4H), 2.95 (s, 2H), 1.35 (s, 3H), 1.31 (t, 3H, J = 7 Hz), 1.20 (bs, 2H), ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 174.0, 141.0, 128.4, 128.0, 126.0, 69.0, 60.0, 55.0, 48.0, 20.0, 14.0. HRMS ($C_{20}H_{26}N_2O_2Na^+$) calculated = 349.1886, observed = 349.1873.

(*S*)-Ethyl-3-amino-2-(dibenzylamino)-2-methylpropanoic acid (17)

An amount of 1.41 g (4.3 mmol) of **16** was dissolved in 25 mL of EtOH. An amount of 0.52 g (12.9 mmol) of well crushed NaOH pellets were added. The solution was heated to reflux solvent for 4 h. The EtOH layer was acidified to pH 2, evaporated to dryness under high vacuum, and triturated with MeOH. The MeOH layer was neutralized with solid $NaHCO_3$, filtered, and evaporated under reduced pressure giving 1.16 g (3.9 mmol, 90%) of **17** as yellowish wax. ^{1}H-NMR (CD_3OD,

400 MHz): δ 7.18 (m, 10H), 3.91 (m, 4H), 2.83 (m, 2H), 1.87 (s, 3H). ^{13}C-NMR (CD_3OD, 100 MHz): δ 182.0, 144.0, 129.6, 129.0, 127.0, 70.0, 56.0, 22.0. HRMS ($C_{18}H_{22}N_2O_2Na^+$) calculated = 321.1573, observed = 321.1573.

Synthesis of (*S*)-2-(dibenzylamino)-3-(*tert*-butyloxycarbonylamino)-2-methylpropanoic acid (18)

A solution of 1 g of **17** (3.4 mmol) in 10 mL water was placed in a 50 mL round bottom flask. An amount of 0.56 g (6.7 mmol) of $NaHCO_3$ (2 eq.) was added with stirring. The solution was cooled to 0 °C. A solution of 0.98 g (4.7 mmol) $(Boc)_2O$ (1.4 eq.) in 10 mL 1,4 dioxane was added drop wise. The reaction was allowed to stir at 0 °C for an hour. The reaction was then allowed to warm to RT overnight. The reaction mixture was diluted with 15 mL H_2O, acidified to pH 4 with $NaHSO_4$, and extracted twice with Et_2O. The combined ether layer was washed with water (5 × 30 mL), washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography (40% EtOAc–hexanes) giving 1.2 g (3 mmol, 91%) of **18** as a white solid. R_f = 0.28 (40% EtOAc–hexane). IR (cm^{-1}) = 2977, 1698, 1494. MP = 58 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.21 (m, 10H), 5.44 (bs, 1H), 4.09 (m, 4H), 3.64 (m, 2H), 1.42 (m, 12H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 175.0, 156.0, 137.0, 128.8, 128.5, 127.7, 79.0, 71.0, 55.0, 44.0, 28.0, 20.0. HRMS [$C_{23}H_{30}N_2O_4Na^+$] calculated = 421.2097, observed = 421.2094.

(*S*)-2-Amino-3-(*tert*-butyloxycarbonylamino)-2-methylpropanoic acid (19)

A solution of 1 g (2.5 mmol) of **18** in 25 mL MeOH was placed in a pressure bottle. An amount of 0.2 g (20% by weight) of Pd-C was added to the bottle. The solution was placed on a Parr shaker hydrogenation apparatus and allowed to shake with 30 psi hydrogen gas for 12 h. The reaction mixture was filtered through a Celite bed to remove the catalyst. The filtrate was evaporated giving **19**. An amount of 0.5 g (2.3 mmol, 92%) of **19** was obtained as a white wax. R_f = 0.3 (5% MeOH–CH_2Cl_2). IR (cm^{-1}) = 2977, 1701, 1602, 1508. MP = 204 °C. ^{1}H-NMR (CD_3OD, 400 MHz): δ 3.44 (s, 2H), 1.47 (m, 12H). ^{13}C-NMR (CD_3OD, 100 MHz): δ 174.0, 158.0, 79.0, 61.0, 46.0, 27.0, 19.0. HRMS ($C_9H_{18}N_2O_4Na^+$) calculated = 241.1159, 241.1158.

Synthesis of (*S*)-2-(9-fluorenylmethyloxycarbonylamine)-3-(*tert*-butyloxycarbonylamino)-2-methylpropanoic acid (20)

An amount of 0.35 g of $NaHCO_3$ (4.1 mmol) was added to a solution of 0.45 g **19** (2.1 mmol) in 15 mL water with stirring. The solution was cooled 0 °C. A solution of 1.1 g Fmoc-Osu (3.2 mmol) in 15 mL 1,4-dioxane was added to the reaction mixture over 20 min. The reaction was allowed to stir at 0 °C for an hour and then at ambient temperature for 12 h. At that point the reaction was diluted with 30 mL of water, acidified to pH 4 with 4 M HCl, extracted (3 × 50 mL) with Et_2O. The combined ether layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by radial chromatography using 5% MeOH–CH_2Cl_2 giving 0.86 g (1.96 mmol, 85%) of **20** as a white solid. R_f = 0.41 (5% MeOH–CH_2Cl_2), IR (cm^{-1}) = 3317, 2974, 1694, 1513. MP = 82 °C. $[\alpha]_D^{22}$ = −10.5 (c = 1, $CHCl_3$), ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.80 (d, 2H, J = 7 Hz), 7.68 (d, 2H, J = 7 Hz), 7.39 (t, 2H, J = 7 Hz), 7.31 (t, 2H, J = 7 Hz), 4.31 (bs, 2H), 4.22 (t, 1H, J = 7 Hz), 3.55 (m, 2H), 1.44 (s, 12H). ^{13}C-NMR (CD_3OD, 100 MHz): δ 159.0, 157.0, 145.4, 145.3, 143.0, 129.0, 128.0, 126.4, 126.3, 121.0, 80.0, 68.0, 55.0, 46.0, 28.0, 21.0. HRMS ($C_{24}H_{28}N_2O_6Na^+$) calculated = 463.1839, observed = 463.1835.

Synthesis of (*S*)-*tert*-butyl 2-*tert*-butyloxyaminobutyl-4-diazo-2-methyl-3-oxobutanoate (21)

Acid **11** (3 g, 8.7 mmol) was dissolved in 10 mL THF and cooled to −25 °C. A measured 1 equivalent of Et_3N (1.2 mL, 8.7 mmol) and 1.05 equivalents of $ClCO_2Me$ (710 μL, 9.1 mmol) was added drop wise to the THF solution. The mixture was stirred for 2 h giving rise to the mixed anhydride, which was taken immediately for the next step. The resulting white suspension of the mixed anhydride was allowed to warm to 0 °C and a solution of dry diazomethane (2 equivalent, 17.4 mmol) in Et_2O was carefully added. The reaction mixture was allowed to stir for 12 h in the dark at 0 °C. Excess diazomethane was removed by passing N_2 through the solution for 30 min. The reaction mixture was then diluted with Et_2O, washed with saturated $NaHCO_3$, saturated NH_4Cl, and brine. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The resulting residue was purified by chromatography (1 : 1 Et_2O–hexanes) giving 2.62 g (7.1 mmol, 82%) of **21** as a clear yellowish oil. R_f = 0.42 (1 : 1 Et_2O–hexanes). IR (cm^{-1}) = 3381, 2976, 2934, 2110, 1704, 1517. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.41 (s, 1H), 4.58 (bs, 1H), 3.09 (bm, 2H), 1.85 (m, 1H), 1.71 (m, 1H), 1.45 (m, 21H), 1.28 (m, 4H). HRMS ($C_{18}H_{31}N_3O_5Na^+$) 392.2156, observed = 392.2153.

(*S*)-3-*tert*-Butyloxyaminobutyl-4-*tert*-butyloxy-3-methyl-4-oxobutanoic acid (22)

An amount of 2.5 g **21** (6.8 mmol) was dissolved in 15 mL 3 : 7 H_2O–THF in a 50 mL round bottom flask. The flask was purged with N_2 and the resulting solution was photolyzed with a Hanovia lamp (500 W) at a distance of approximately 10 cm. The photolysis was allowed to proceed for 48 h. At that point the reaction was found to be completed as evident by TLC. The clear and colorless solution was concentrated under reduced pressure and the water layer was extracted three times with Et_2O. The combined Et_2O layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by chromatography (30% EtOAc–hexanes) giving 1.83 g of **22** (5.1 mmol, 75%) as a yellowish oil. The ^{1}H-NMR and the HRMS is highly indicative of the product. Hence, the product was taken for the next step without further purification. R_f = 0.18 (30% EtOAc–hexanes), IR (cm^{-1}) = 3364, 2977, 2936, 1709, 1521. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 4.54 (bs, 1H), 3.10 (bm, 2H), 2.73 (m, 1H), 2.38 (m, 1H), 1.55 (m, 22H), 1.20 (m, 5H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 176.2, 175.2, 155.9, 80.6, 72.4, 44.4, 42.4, 40.1, 38.8, 30.0, 28.4, 27.8, 21.8, 21.4. HRMS ($C_{18}H_{33}NO_6Na^+$) calculated = 382.2200, observed = 382.2196.

(*S*)-*tert*-Butyl-2-*tert*-butyloxycarbonylaminobutyl-3-(9-fluorenylmethyloxycarbonylamino)-2-methylpropanoate (23)

A volume of 0.79 mL Et_3N was added to a solution of 1.7 g of **22** (4.7 mmol) in 25 mL dichloroethane under N_2 atmosphere. A volume of 1.2 mL (5.2 mmol) DPPA was added to the reaction mixture. The reaction was allowed to stir at RT for 2 h. The mixture was heated to reflux solvent for 3 h. The mixture was cooled and the organic layer was extracted with saturated NH_4Cl solution, dried over $MgSO_4$, and evaporated under reduced pressure giving the isocyanate. The isocyanate was taken up in dry toluene under N_2 atmosphere. An amount of 1.84 g (9.4 mmol) 9-fluorenylmethanol was added to the solution along with 100 μL of Ti(IV) isopropoxide. The reaction was heated to 80 °C over night. The reaction was cooled and the organic layer was evaporated under reduced pressure. The residue was then purified by chromatography (CH_2Cl_2 to 3% MeOH–CH_2Cl_2) giving 2 g **23** (3.76 mmol, 80%) as sticky light yellowish wax. The product was found too sticky to dry the solvent all the way. It was characterized by ^{1}H-NMR and HRMS. The product was taken for the next step without further attempt to purify it. R_f = 0.78 (3% MeOH–CH_2Cl_2). IR (cm^{-1}) = 3340, 2975, 2933, 1756, 1688, 1513. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.76 (d, 2H, J = 7 Hz), 7.59 (d, 2H, J = 7 Hz), 7.39 (t, 2H, J = 7 Hz), 7.30 (t, 2H, J = 7 Hz), 4.60 (bm, 2H, J = 7 Hz), 4.37 (m, 2H), 4.22 (t, 1H, J = 7 Hz), 3.38 (m, 1H), 3.24 (m, 1H), 3.09 (m, 2H), 1.46 (bm, 6H), 1.44 (bm, 21H). HRMS ($C_{32}H_{44}N_2O_6Na^+$) calculated = 575.3091, observed = 575.3083.

Synthesis of (*S*)-Fmoc-α-methyl-β^{2,2}-lysine-Boc-OH (24)

An amount of 1.5 g of **23** (2.7 mmol) was dissolved in 20 mL of 1 : 1 TFA–CH_2Cl_2. The solution was allowed to stir for 12 h at RT under N_2 atmosphere. The reaction was monitored by TLC (5% MeOH–CH_2Cl_2) and ESI-mass spectrometry for the completion. At which point the TFA–CH_2Cl_2 layer was evaporated under reduced pressure giving free amino acid. The residue was taken up in 15 mL of H_2O and 0.54 g (6.5 mmol) $NaHCO_3$ was added to the solution slowly to control the effervescence. The mixture was cooled to 0 °C. A solution of 0.71 g $(Boc)_2O$ (3.2 mmol) in 15 mL 1,4-dioxane was added to the mixture slowly at 0 °C. The reaction was allowed to stir at 0 °C for an hour. The reaction was then allowed to warm to RT and stir for 12 h. The reaction mixture was extracted with pentane to remove excess $(Boc)_2O$. The aqueous layer was then acidified to pH 4 with 2 N HCl, extracted three times with Et_2O (50 mL). The combines ether layer was dried over MgSO4, evaporated under reduced pressure, purified by chromatography (5% MeOH–CH_2Cl_2) giving 1.21 g of **24** (2.44 mmol, 90% over two steps) as a white solid after purification by flash chromatography (CH_2Cl_2 to 5% MeOH–CH_2Cl_2). R_f = 0.50 (5% MeOH–CH_2Cl_2). IR (cm^{-1}) = 3338, 2940, 1693, 1518. $[\alpha]_D^{23}$ = −6.0 (c = 0.7, $CHCl_3$). MP = 73 °C. ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.81 (d, 2H, J = 7 Hz), 7.66 (d, 2H, J = 7 Hz), 7.40 (t, 2H, J = 7 Hz), 7.32 (t, 2H, J = 7 Hz), 4.36 (m, 2H), 6.23 (m, 1H), 3.03 (t, 2H, J = 7 Hz), 1.61 (m, 1H), 1.44 (m, 12H), 1.30 (m, 2H), 1.13 (s, 3H). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.75 (d, 2H, J = 7 Hz), 7.58 (m, 2H), 7.38 (t, 2H, J = 7 Hz), 7.30 (t, 2H, J = 7 Hz), 6.37 (bm, 1H), 5.42 (bm, 1H), 4.58 (m, 1H), 4.35 (m, 1H), 4.21 (m, 1H), 3.36 (m, 2H), 3.09 (bm, 2H), 1.41 (m, 18H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 179.5, 156.9, 156.2, 143.6, 141.2, 127.7, 127.1, 125.1, 120.0, 79.3, 66.8, 47.3, 46.8, 40.0, 36.7, 36.0, 30.4, 28.4, 21.3, 20.4. HRMS ($C_{28}H_{36}N_2O_6Na^+$) calculated = 519.2965, observed = 519.2459.

Synthesis and specific binding of Vapreotide analogue (25) against IMR 32 cell line

The Vapreotide analogue (**25**) was synthesized in collaboration with New England Peptide (Gardner, MA) using properly protected (*S*)-α^{2,2}-methyllysine analogue (**7**) prepared in our laboratories as described above. The Vapreotide analogue was determined to be 99% pure by HPLC.

Binding of the Vapreotide analogue (**25**) was conducted against IMR 32 human neuroblastoma cells. These cells over express SSTR2 receptors. In order to perform the binding assays four groups of triplicate wells were studied (n = 12 total). Each well contained 500 000 IMR 32 cells in 2 mL of media. These wells also contained 100 000 counts of ^{111}In-pentetreotide. Three wells were competed with 10^{-6} M octreotide and three wells were competed with **25**. All 12 wells were incubated at 37 °C for 20 hours. Cells were harvested, washed and counted in a gamma counter. However, gamma counter result revealed that the Vapreotide analogue **25** has no specific binding for SSTR2 (Table 2 in ESI†).

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