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Comparison of the Inhibition of Human and *Trypanosoma cruzi* Prolyl Endopeptidases

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Abstract—Prolyl endopeptidases (PEPs) have been found in numerous species. Inhibitors of human enzyme could correct cognitive deficits in Alzheimer patients while inhibition of *Trypanosoma cruzi* PEP could prevent invasion phase in Chagas disease. A structure–activity relationship study carried out in a tetrahydroisoquinoline series allowed to obtain potent competitive inhibitors superior to SUAM-1221. Besides, inhibitors expected to act according to an irreversible mechanism revealed to be superior to JPT-4819, for applications linked to human enzyme inhibition.

Introduction

Prolyl oligopeptidase (prolyl endopeptidase, post-proline cleaving enzyme, PEP, EC 6.4.21.26) is a proline-specific endopeptidase with a serine-type mechanism, cleaving peptide bonds on the carboxylic side of prolyl residues.[1] This enzyme was first isolated in the human uterus,[2] and has later been identified and purified from various mammalian tissues.[3–6] In the central nervous system, PEP degrades proline-containing neuropeptides involved in the processes of learning and memory, such as vasopressin, substance P and thyrotropin-releasing hormone (TRH).[7] Therefore, as cognitive deficits in Alzheimer patients are reported to show improvement with TRH, one can postulate that PEP inhibitors could prevent memory loss in patients suffering of senile dementia. Interestingly, the enzyme was found to be exclusively active on small peptides, which further stresses the specificity of this particular enzyme.

PEPs have also been found in other species such as fungi, bacteria and plants.[8,9] We have reported that *Trypanosoma cruzi*, the causative agent of Chagas disease, also contains a prolyl endopeptidase which exhibits the unusual property of cleaving, in addition to the small peptides, collagens of the extracellular matrix.[10] These data suggest that the inhibition of this proline-specific enzyme may represent a therapeutic approach to the treatment of Chagas disease, by preventing the invasion of mammalian cells by trypomastigotes.

While most of the PEP inhibitors described in the literature are structurally related to Z-prolyl-prolinal,[11] the collagenase-like substrate Gly-Pro-Leu-Gly-Pro was also identified as a lead molecule.[10] To obtain new leads against both human and *T. cruzi* PEPs, two orthogonal peptide combinatorial libraries were screened and led to a 1,2,3,4-tetrahydroisoquinoline carboxylic acid (Tic) derivative, active in the low micromolar range against the *T. cruzi* enzyme.[12,13] Subsequently, the automated parallel synthesis and the screening of a focused Tic-based library allowed us to select lead **1** (Fig. 1), which displayed an IC_{50} of 7 nM against the parasitic PEP while its activity towards human PEP was found to be less potent ($IC_{50} = 550$ nM).[14] Here, we report our efforts to establish structure–activity relationships based upon lead **1** and to obtain more potent inhibitors against human and *T. cruzi* PEPs. For the latter, obtaining a good selectivity index was an additional aim.

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Chemistry

The starting materials Boc-L-Tic-pyrrolidine, Boc-L-Tic-thiazolidine, Boc-L-Tic(7-OH)-pyrrolidine and Boc-L-Tic-L-Pro-OCH_3 were obtained by reacting Boc-L-Tic or 7-OH-Boc-L-Tic(7-OH) with pyrrolidine, thiazolidine or L-Pro-OCH_3, using DCC/HOBt as coupling reagents in dichloromethane (Scheme 1). Boc-L-Tic(7-OH) was prepared from L-di-iodo-Tyr according to Verschueren et al.[15] All inhibitors were then prepared in solution phase from Boc-L-Tic-pyrrolidine (**1** and **6**–**26**), Boc-L-Tic-thiazolidine (**2**–**3**) Boc-L-Tic(7-OH)pyrrolidine (**4**–**5**) or Boc-L-Tic-L-Pro-OCH_3 (**27**–**31**). After TFA deprotection, each terminal group was substituted by reaction with the appropriate carboxylic acid (**1-**–**26**) using the conditions described previously (Scheme 1).

In the case of compounds **27**–**30**, the methyl ester obtained from Boc-L-Tic-L-Pro-OCH_3 was transformed to the chloromethylketone **28** according to Kowalski et al. using LDA/CH_2ICl as reagents (Scheme 2).[16] Treatment with potassium acetate in DMF led to the corresponding acetyloxymethylketone **29**, which was hydrolysed into the hydroxymethylketone **30** with Na_2CO_3 in MeOH (Scheme 2). The methyl ester also led to the aldehyde **27** as a racemic mixture by treatment with DIBAL-H (Scheme 2).

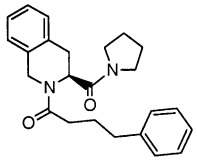
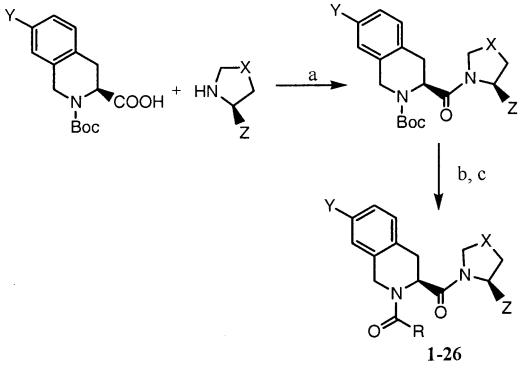


Figure 1. Structure of compound **1**.



Scheme 1. Synthesis of compounds **1**–**26**. Reagents: (a) DCC/HOBt, CH_2Cl_2; (b) TFA/CH_2Cl_2 50/50; (c) RCOOH, DCC/HOBt, CH_2Cl_2.

In the case of the nitrile derivative **31**, 4-phenyl-butyryl-L-Tic was coupled to Pro-NH_2 before dehydratation according to Pozdnev et al. using trifluoroacetic anhydride in THF (Scheme 3).[17]

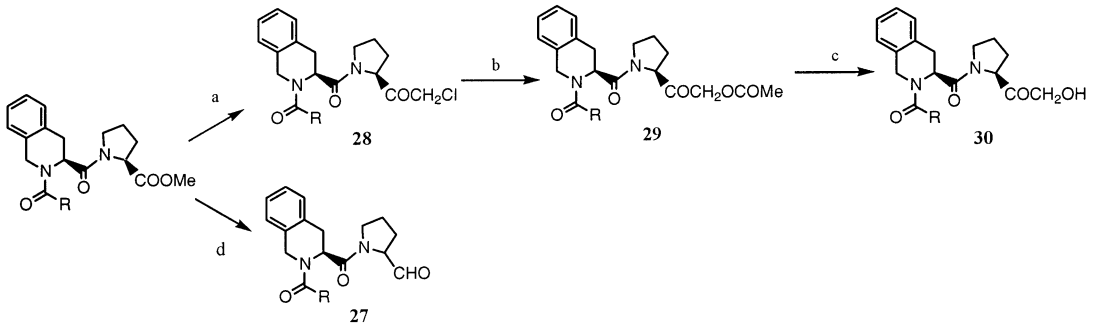
Assays of PEPs inhibition

T. cruzi PEP was purified following two steps of chromatography (DEAE and phenylsepharose) as previously described.[10] Enzyme inhibition was evaluated by using the fluorogenic substrate Suc-Gly-Pro-Leu-Gly-Pro-AMC ($K_m = 12.5$ μM). The test compound was dissolved at various concentrations in Tris–HCl buffer (25 mM, pH 7.5), containing 10% DMSO. 20 μL of the solution was preincubated for 15 min with 20 μL of enzyme in the same buffer at 37 °C. The enzymic reaction was then initiated by adding 20 μL of the substrate solution (33 μM). After 15 min, the reaction was stopped by adding 100 μL of EtOH. The fluorescence of free released AMC was measured at 440 nm upon excitation at 380 nm in a Hitachi 2000 spectrofluorimeter. Inhibitory potency was evaluated by the IC_{50} value, which was defined as the concentration of the test compound that resulted in 50% inhibition of the fluorescence with respect to the DMSO control.

Human PEP was purified from human platelets following two steps of chromatography (DEAE and phenylsepharose) and enzyme activity was measured as previously reported.[18,19] At 100 μL of K-phosphate buffer (100 mM, pH 7.5) containing 1 mM EDTA, NaN_3 and DTT, was added 5 μL of inhibitor in DMSO at various concentrations and 20 μL of enzyme. After 20 min at 37 °C, the reaction was started by addition of 5 μL of substrate (Z-Gly-Pro-AMC, $K_m = 120$ μM, 4.3 mM dissolved in 40% DMSO). After 15 min, the reaction was stopped by adding 500 μL of acetic acid and the fluorescence was measured in the same conditions as for the *T. cruzi* assay.

Results

Modifications were successively introduced at the three binding subsites named P1, P2 and P3 according to the



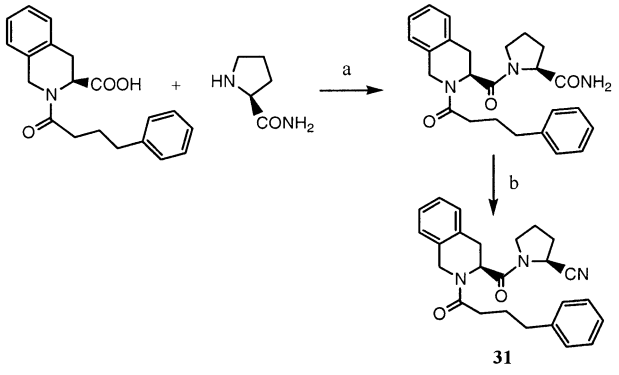
Scheme 2. Synthesis of compounds **27**–**30**. Reagents: (a) LDA/CH_2ICl, THF, −78 °C; (b) CH_3COOK, DMF, 60 °C; (c) Na_2CO_3, MeOH; (d) DIBALH, THF, −78 °C.

Schechter–Berger nomenclature.[20] Activity upon *T. cruzi* and human PEPs are given in Tables 1–3, respectively, for compounds **1**–**9**, **10**–**26** and **27**–**31**.

While introduction of a sulphur atom in the pyrrolidine ring (subsite P1) was reported to be very favourable towards bovine brain PEP inhibition,[21,22] only a slight increase of activity was observed towards parasitic and human PEPs compared to lead **1** ($IC_{50}=5$ and 120 nM respectively for compound **2**) which was however associated with a decrease of selectivity. The corresponding sulphone derivative **3** was found to be less active ($IC_{50}=90$ and 2800 nM). Addition of a hydroxyl group in the C-7 position of the Tic ring (subsite P2, compound **4**), was favourable towards human enzyme ($IC_{50}=70$ nM) and slightly unfavourable towards parasitic enzyme ($IC_{50}=19$ nM). Ethyl ether derivative (**5**) showed a similar potency upon *T. cruzi* enzyme compared to the hydroxyl derivative **4**, while showing a better selectivity.

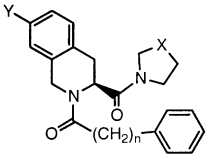
Optimization of the subsite P3 was approached according to two types of modifications: (i) replacement of the phenyl ring by heterocyclic, polyaromatic, alicyclic or isosteric structures and (ii) variation of the distance to subsite P2. Reverse changes in the electronic density of the phenyl ring (**10**–**11**) or replacement of the phenyl ring with an isostere such as thiophene (**12**) led to an increase of activity against human PEP with, however, a decrease of selectivity towards *T. cruzi* PEP. Steric hindrance decreased activity against parasitic enzyme (**13**). Whatever the distance to subsite P2, the presence of a nitrogen atom in a phenyl ring was unfavourable for both PEPs (**14**–**15** versus **6**–**7**).

The potent inhibition found for compound **16** against *T. cruzi* PEP within the Tic-based library ($IC_{50}=9$ nM; 7 and 12 nM for its separated isomers),[14] led us to synthetize and evaluate activities of its analogues **17**–**26**. Results underline the good recognition by the parasitic enzyme of the cyclohexyl ring, substituted or not (**16**



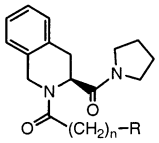
Scheme 3. Synthesis of compound **31**. Reagents: (a) DCC/HOBt, CH_2Cl_2; (b) $(CF_3CO)_2O$, THF.

Table 1. Activity of compounds **1**–**9** towards *T. cruzi* and human PEPs



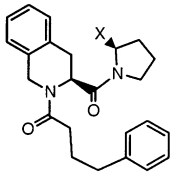
Compd	X	Y	*n*	*T. cruzi* PEP IC_{50} (nM)	Human PEP IC_{50} (nM)	Selectivity index IC_{50} human PEP/IC_{50} *T. cruzi* PEP
1	CH_2	H	3	7	550	78
2	S	H	3	5	120	24
3	SO_2	H	3	90	2800	31
4	CH_2	OH	3	19	70	3.7
5	CH_2	OC_2H_5	3	17	125	7.3
6	CH_2	H	0	1300	4000	3.1
7	CH_2	H	1	105	400	3.8
8	CH_2	H	2	525	850	1.6
9	CH_2	H	4	15	100	6.6

Table 2. Activity of compounds **10**–**23** towards *T. cruzi* and human PEPs



Compd	R	*n*	*T. cruzi* PEP IC_{50} (nM)	Human PEP IC_{50} (nM)	Selectivity index
10	4-Nitrophenyl	3	52	220	4.2
11	4-Methoxyphenyl	3	54	150	2.7
12	Thiophen-2-yl	3	10	55	5.5
13	Pyren-1-yl	3	100	50	0.5
14	Pyridin-3-yl	1	1200	600	0.5
15	Pyridin-3-yl	0	3500	34,000	9.7
16	4-Methylcyclohexyl	1	9	16	1.7
17	4-Methylcyclohexyl	0	370	1030	2.8
18	Cyclohexyl	0	395	600	1.5
19	Cyclohexyl	1	12	175	14.6
20	Cyclohexyl	2	105	150	1.4
21	Cyclohexyl	3	150	70	0.5
22	Cyclohexyl	4	>500	370	0.5
23	Cyclopentyl	1	67	400	5.9
24	Norborn-2-yl	1	370	1750	4.7
25	Adamant-1-yl	1	115	300	2.6
26	Dicyclohexylmethyl	0	6500	1500	0.2

Table 3. Activity of compounds **27**–**31** towards *T. cruzi* and human PEPs



Compd	X	*T. cruzi* PEP IC_{50} (nM)	Human PEP IC_{50} (nM)
27	CHO	12	Nd
28	$COCH_2Cl$	2.6	1.5
29	$COCH_2OCOCH_3$	3.9	3.5
30	$COCH_2OH$	4.5	1.5
31	CN	11	40
	JTP-4819	16	3.6

and **19**) and the influence of the length of the spacer: the best activities were obtained for one methylene group. However, except for compound **19**, no or a weak selectivity between both enzymes was noted in this series. Influence of the length of the spacer was also observed in the phenyl series (**6**–**9**): the best inhibition activity towards *T. cruzi* PEP associated to the best selectivity was obtained for a spacer of three methylene groups.

Derivatives corresponding to additional reactive group in compound **1** (**27**–**31**) were found to be very active towards both enzymes with IC_{50} values in the low nanomolar range (Table 3).

Discussion

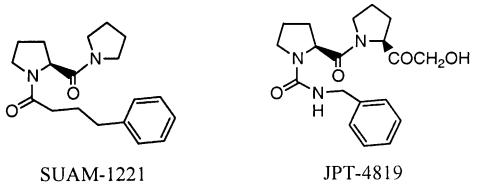
In the aim of discovering new potent inhibitors against *T. cruzi* and human PEPs, the screening of two orthogonal D-tripeptide combinatorial libraries and of a focused Tic-based library were carried out. They led to the selection of compound **1** displaying IC_{50} of 7 and 550 nM against parasitic and human enzymes, respectively. The three parts of the molecule P1, P2 and P3 as well as the spacer between P2 and P3 were successively modified to analyse their influence in the recognition of the corresponding enzyme subsites S1, S2 and S3. These latters have been recently well defined in the case of porcine PEP co-crystallised with the Z-prolyl-prolinal inhibitor.[23] They were assumed identical in *T. cruzi* and human PEPs because of the high sequence similarity between PEPs of different organisms, particularly in the region containing the catalytic triad.[24] All reported results so far, and those deduced from our focused Tic-based library (32 amines tested), converge to point out pyrrolidine ring as the most appropriate subsite P1 for PEPs recognition, whatever their origin.

Pyrrolidine ring presents the optimal size to fit into the hydrophobic pocket S1, defined by residues Trp595, Phe476, Val644, Tyr599 and Asn555, in the case of porcine PEP, the only crystallised PEP described so far,[23] and its replacement with other close rings such as piperidine or azetidine, strongly decreases inhibition.[21] Its replacement for a thiazolidine ring (**2**) improves activity towards human PEP but at a lesser extent compared to the bovine brain PEP where a 20-fold increase was observed for similar replacements in a proline series.[22]

Conversely, the intermediate subsite P2 allows a widest diversity and replacement of proline and thiaproline for unnatural analogues such as perhydroindole, azabicyclo[2.2.2]- and azabicyclo[2.2.1]-heptane led to 10/20-fold increases of activity against rat brain PEP.[21] In the same way, replacement of proline by the Tic analogue as in compound **1**, increases the activity against *T. cruzi* PEP (data not shown). In all cases, stereochemistry of the central moiety was important for recognition,[21,22] for example, IC_{50} value for the D-Tic isomer of lead compound **1** against *T. cruzi* PEP, was 200 nM instead of 7 nM for the L-Tic isomer. Introduction of an hydroxyl group or an alkoxy group in the C-7 position of the Tic ring (**4**–**5**) in the aim of modulating bioavailability of inhibitors by addition of hydrophilic or hydrophobic side chains, maintains or enhances activity, compared to compound **1**, respectively against parasitic and human PEP. This result increases the interest of the Tic residue as a P2 subsite.

In accordance with previous reports, good activities are found with the alicyclic moieties as well as with the aromatic ring chosen as subsite P3.[25,26] These results can be explained by the interaction with the hydrophobic environment of the subsite S3, due to the presence of residues Phe173, Met235, Cys255, Ile591 and Ala594.[23] For the same reason, it is not surprising that a variation of electronic density in the ring does not lead to significant difference of inhibitory potency (**10**–**11**). However, length of the spacer has a different influence according to each series. Recognition in the phenyl series is optimal when aromatic ring and Tic residue are linked by three methylene groups while the cyclohexyl ring has to be closer, result reinforcing those obtained with an open chain.[27]

Except for the slight increase of activity observed by introduction of a sulphur atom in the pyrrolidine ring, compound **1** remained the most potent inhibitor and was therefore modified by reactive groups likely to lead from a competitive inhibition to a tight-binding or an irreversible mode of action. The first mechanism could be involved by addition of an aldehyde group capable of generating a tetrahedric complex with the catalytic serine, which could mimic the transition state proved from the co-crystallisation porcine PEP/Z-prolyl-prolinal.[23] The aldehyde derivative **27** obtained under the racemic form, was only tested upon the parasitic enzyme and gave IC_{50} value in the low nanomolar range (12 nM). The possibility of an irreversible mode of action was also considered via the preparation of methylketones substituted by good leaving groups. The groups chosen, chloromethylketone, acetyloxymethyl ketone and hydroxymethylketone, led to IC_{50} values below 4.5 nM towards both enzymes (**28**–**30**). Compounds were found to effectively act on *T. cruzi* PEP as irreversible inhibitors or very tight-binding inhibitors since less than 1% of enzymic activity was recovered following extensive dialysis. Therefore, it can be expected that they act on the human PEP in a similar way. It can be noted that the hydroxymethylketone **30** displays a 2-fold decrease of the IC_{50} value (1.5 nM) compared to JPT-4819 (Table 3 and Fig. 2), previously reported as capable to improve memory impairment in rat by activating cerebral peptidergic and cholinergic neurons via inhibition of PEP.[28] Besides, nitrile derivative **31**, likely to behave as a non-competitive reversible inhibitor,[29,30] yields to a more moderate increase of activity upon human enzyme.



SUAM-1221 JPT-4819

Figure 2. Structure of SUAM-1221 and JPT-4819.

In conclusion, the structure–activity relationship study carried out in this 1,2,3,4-tetrahydroisoquinoline series allowed us to obtain competitive inhibitors superior to phenylpropylcarbonyl-Pro-pyrrolidine known as SUAM-1221 (Fig. 2), a PEP inhibitor used as control in the literature against PEPs.[20] Besides, inhibitors expected to act according to an irreversible mechanism revealed themselves to be superior to JPT-4819 for applications linked to human enzyme inhibition. For the latter applications, compared to proline analogues, presence of the hydrophobic Tic residue at the subsite P2, constitutes a major advantage for the blood–brain barrier penetrability.

Experimental

Chemistry

All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) using UV light as a visualising agent. Chromatography was undertaken using silica gel 60 (230–400 mesh ASTM) from Macherey-Nagel. Thick-layer chromatography (TLC) was performed using silica gel from Merck, from which the compounds were extracted by the following solvent system: CH_2Cl_2/MeOH, 85:15. ^1H NMR spectra were obtained using a Bruker 300 MHz spectrometer, chemical shifts (δ) were expressed in ppm relative to TMS used as an internal standard. Mass spectra were recorded on a time-of-flight (TOF) plasma desorption spectrometer using a Californium source. The purity of final compounds was verified by high pressure liquid chromatography (HPLC) with a C18 vydac column. Analytical HPLC was performed on a Shimadzu system equiped with a UV detector set at 254 nm. Compounds were dissolved in EtOH and injected through a 50 μL loop. The following eluent systems were used: A (H_2O/TFA, 100:0.05) and B (CH_3CN/H_2O/TFA, 80:20:0.05). HPLC retention times (HPLC t_R) were obtained, at flow rates of 1 mL/min, using the following conditions: a gradient run from 100% eluent A during 5 min, then to 100% eluent B over the next 25 min. L-Tic was obtained from Lancaster, 3,5-diiodo-L-Tyr, $2H_2O$ from Acros, coupling reagents from Novabiochem and other reagents from Acros, Aldrich or Lancaster.

Method A. General procedure for the protection of amines by a Boc group. To a solution of the amine (30 mmol, 1 equiv) in 120 mL of a H_2O/dioxane/NaOH 1 M 1:2:1 mixture, was added, at 0 °C, Boc_2O (33 mmol, 1.1 equiv). After stirring the mixture at room temperature for 5 h, the solvent was evaporated with the residue being taken up in 30 mL of AcOEt and washed with 30 mL of aqueous citric acid 10% and 30 mL of brine. The organic layer was separated, dried over $MgSO_4$ and the solvent evaporated to yield the desired product.

Method B. General procedure for the coupling reaction. At 0 °C, to a solution of the carboxylic derivative (1.4 mmol, 1 equiv) in 10 mL of CH_2Cl_2, were added HOBt (1.4 mmol, 1 equiv) in 2 mL of DMF and DCC (1.4 mmol, 1 equiv) in 2 mL of CH_2Cl_2, and, after 10 min, the amine (1.4 mmol, 1 equiv). After stirring the mixture at room temperature for 15 h, the solvent was evaporated and the residue taken up in 50 mL of AcOEt, cooled at −20 °C, filtered then washed with 40 mL of Na_2CO_3 1 M, 40 mL of aqueous citric acid 20% and 30 mL of brine. The organic layer was separated, dried over $MgSO_4$, the solvent evaporated and the residue purified by TLC (CH_2Cl_2/MeOH, 95:5 to 90:10) to yield the desired product.

Method C. General procedure for deprotection of the Boc group followed by a coupling reaction. The Boc-aminoacid (1.4 mmol, 1 equiv) was diluted in 10 mL of a TFA/CH_2Cl_2 1:1 mixture. After stirring the mixture at room temperature for 1 h, the solvent was evaporated and the residue taken up in 10 mL of CH_2Cl_2 with an excess of DIEA (7.0 mmol, 5 equiv). To this solution were added HOBt (1.4 mmol, 1 equiv) in 2 mL of DMF and DCC (1.4 mmol, 1 equiv) in 2 mL of CH_2Cl_2, then, after 10 min, the amine (1.4 mmol, 1 equiv). After stirring the mixture at room temperature for 15 h, the solvent was evaporated with the residue being taken up in 50 mL of AcOEt, cooled at −20 °C, filtered then washed with 40 mL of Na_2CO_3 1 M, 40 mL of aqueous citric acid 20% and 30 mL of brine. The organic layer was separated, dried over $MgSO_4$, the solvent evaporated and the residue purified by TLC (CH_2Cl_2/MeOH, 97:3 to 90:10) to yield the desired product.

Boc-L-Tic. Boc-L-Tic was prepared from L-Tic (1 g, 5.6 mmol, 1 equiv) by method A and was obtained as a white solid (1.36 g, 88% yield); R_f 0.60 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 20.27 min; ^1H NMR (DMSO-d_6) δ 1.36 + 1.42 (s, 9H, $C(CH_3)_3$ *cis*/*trans*), 2.99–3.15 (m, 2H, Tic–H), 4.32–4.62 (m, 2H, Tic–H), 4.82–4.85 (m, 1H, Tic–H), 7.14–7.16 (m, 4H, Ar–H), 12.60 (s, 1H, COOH); TOFMS *m*/*z* 177 (M^+ − Boc).

Boc-L-Tic-pyrrolidine. Boc-L-Tic-pyrrolidine was prepared from Boc-L-Tic (1 g, 3.6 mmol, 1 equiv) and pyrrolidine (300 μL, 3.6 mmol, 1 equiv) by method B and was obtained after TLC (CH_2Cl_2/MeOH, 90:10) as a white solid (1.18 g, 95% yield); R_f 0.85 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 21.93 min; ^1H NMR ($CDCl_3$) δ 1.42 + 1.48 (s, 9H, $C(CH_3)_3$ *cis*/*trans*), 1.82–2.04 (m, 4H, pyrrolidine), 3.01–3.09 (m, 2H, Tic–H), 3.38–3.65 (m, 4H, pyrrolidine), 4.34 + 4.52 (d, J = 15.3 + 16.1 Hz, 1H, Tic–H *cis*/*trans*), 4.53–4.57 + 5.02–5.05 (m, 1H, Tic–H *cis*/*trans*), 4.82 + 4.96 (d, J = 15.3 + 16.1 Hz, 1H, Tic–H *cis*/*trans*), 7.03–7.33 (m, 4H, Ar–H); TOFMS *m*/*z* 330 (M^+).

4-Phenylbutyryl-L-Tic-pyrrolidine (1). Compound **1** was prepared from Boc-L-Tic-pyrrolidine (695 mg, 3.0 mmol, 1 equiv) and 4-phenylbutyric acid (405 mg, 3.0 mmol, 1 eqiv.) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 95:5) as a colourless oil (895 mg, 79% yield); R_f 0.55 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 22.94 min; ^1H NMR ($CDCl_3$) δ 1.82–2.04 (m, 6H, $CH_2\underline{CH_2}CH_2C_6H_5$ and pyrrolidine), 2.43–2.49 (m, 2H, $\underline{CH_2}CH_2CH_2C_6H_5$), 2.68 (t, J = 7.3 Hz, 2H, $CH_2CH_2\underline{CH_2}C_6H_5$), 3.07 (d, J = 6.5 Hz, 2H, Tic–H), 3.35–3.53 (m, 4H, pyrrolidine), 3.74–3.80 (m, 1H, pyr-

rolidine), 4.55 (d, $J=15.3$ Hz, 1H, Tic–H), 4.64 (d, $J=15.3$ Hz, 1H, Tic–H), 5.16 (t, $J=6.5$ Hz, 1H, Tic–H), 7.05–7.30 (m, 9H, Ar–H); TOFMS m/z 376 (M^+).

Boc-L-Tic-thiazolidine. Boc-L-Tic-thiazolidine was prepared from Boc-L-Tic (255 mg, 0.93 mmol, 1 equiv) and thiazolidine (73 μL, 0.93 mmol, 1 equiv) by method B and was obtained after TLC (CH_2Cl_2/MeOH, 95:5) as a colourless oil (284 mg, 88% yield); R_f 0.90 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 21.99 min; ^{1}H NMR ($CDCl_3$) δ 1.28 + 1.34 (s, 9H, $C(CH_3)_3$ *cis*/*trans*), 2.69–3.06 (m, 4H, Tic–H and thiazolidine), 3.83–3.85 (m, 2H, thiazolidine), 4.19–5.00 (m, 5H, Tic–H and thiazolidine), 7.00–7.10 (m, 4H, Ar–H); TOFMS m/z 248 (M^+ −Boc).

4-Phenylbutyryl-L-Tic-thiazolidine (2). Compound **2** was prepared from Boc-L-Tic-thiazolidine (202 mg, 0.8 mmol, 1 equiv) and 4-phenylbutyric acid (135 mg, 0.8 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 95:5) as a colourless oil (245 mg, 77% yield); R_f 0.75 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 23.55 min; ^{1}H NMR ($CDCl_3$) δ 1.97 (qt, $J=6.8$ Hz, 2H, $CH_2\underline{CH_2}CH_2C_6H_5$), 2.41–2.47 (m, 2H, $\underline{CH_2}CH_2CH_2C_6H_5$), 2.66 (t, $J=7.4$ Hz, 2H, $CH_2CH_2\underline{CH_2}C_6H_5$), 2.86–3.07 (m, 4H, Tic–H and thiazolidine), 3.71–4.05 (m, 2H, thiazolidine), 4.46–4.71 (m, 4H, Tic–H and thiazolidine), 5.15–5.19 (m, 1H, Tic–H), 7.03–7.28 (m, 9H, Ar–H); TOFMS m/z 394 (M^+).

4-Phenylbutyryl-L-Tic-thiazolidin-1-sulfone (3). To a solution of compound **2** (120 mg, 0.3 mmol, 1 equiv) in 10 mL of CH_2Cl_2 dried on molecular sieves, was added *meta*-chloroperbenzoic acid (265 mg, 1.5 mmol, 5 equiv). After stirring the mixture at room temperature for 18 h, the solution was diluted in 20 mL of CH_2Cl_2, washed with 20 mL of a saturated solution of $NaHCO_3$ containing 10% of sodium sulfite. The organic layer was dried over $MgSO_4$, the solvent evaporated and the residue purified by TLC (CH_2Cl_2/MeOH, 95:5) to yield compound **3** as a colourless oil (90 mg, 70% yield); R_f 0.75 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 22.00 min; ^{1}H NMR ($CDCl_3$) δ 1.91–2.01 (m, 2H, $CH_2\underline{CH_2}CH_2C_6H_5$), 2.41–2.47 (m, 2H, $\underline{CH_2}CH_2CH_2C_6H_5$), 2.66 (t, $J=7.4$ Hz, 2H, $\underline{CH_2}CH_2CH_2C_6H_5$), 3.06 (d, $J=6.4$ Hz, 2H, Tic–H), 3.26–3.40 (m, 2H, thiazolidine), 4.00–4.05 (m, 2H, thiazolidine), 4.41–4.49 (m, 2H, thiazolidine), 4.50 (d, $J=15.4$ Hz, 1H, Tic–H), 4.57 (d, $J=15.4$ Hz, 1H, Tic–H), 5.15–5.22 (m, 1H, Tic–H), 7.05–7.30 (m, 9H, Ar–H); TOFMS m/z 426 (M^+).

7-OH-6,8-Diiodo-L-Tic. To a solution of 3,5-diiodo-L-Tyr, $2H_2O$ (10 g, 22 mmol, 1 equiv) in 100 mL of chlorhydric acid 35%, were added 1,2-dimethoxyethane (7.3 mL) and formaldehyde (7 mL, 88.4 mmol, 4 equiv). After stirring the mixture at 72 °C for 30 min, were added chlorhydric acid 35% (44.2 mL), 1,2-dimethoxyethane (3.7 mL) and formaldehyde (3.5 mL, 44.2 mmol, 2 equiv). After further stirring of the mixture at 72 °C for 18 h, the solution was cooled in an ice bath and filtered, the residue washed with 3×5 mL of 1,2-dimethoxyethane and dried under vacuum to yield 7-OH-6,8-diiodo-L-Tic as a white solid (5.4 g, 55% yield); R_f 0.45 (CH_2Cl_2/MeOH, 8:2); HPLC t_R 11.57 min; ^{1}H NMR (DMSO-d_6) δ 3.02 (dd, $J=10.9$, 16.6 Hz, 1H, Tic–H), 3.16 (dd, $J=4.8$, 16.8 Hz, 1H, Tic–H), 3.95 (d, $J=16.4$ Hz, 1H, Tic–H), 4.04 (d, $J=16.4$ Hz, 1H, Tic–H), 4.26 (dd, $J=4.8$, 10.9 Hz, 1H, Tic–H), 7.65 (s, 1H, Ar–H), 9.63 (broad s, 1H, OH), 10.00 (broad s, 1H, COOH); TOFMS m/z 445 (M^+).

7-OH-6,8-Diiodo-Boc-L-Tic. 7-OH-6,8-Diiodo-Boc-L-Tic was prepared from 7-OH-6,8-diiodo-L-Tic (5.6 g, 12.6 mmol, 1 equiv) by method A and was obtained as a yellow solid (5.8 g, 78% yield); R_f 0.90 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 23.07 min; ^{1}H NMR (DMSO-d_6) δ 1.35 + 1.41 (s, 9H, $C(CH_3)_3$ *cis*/*trans*), 2.99 (m, 2H, Tic–H), 4.14 (d, $J=17.4$ Hz, 1H, Tic–H), 4.36–4.42 (m, 1H, Tic–H), 4.62–4.76 (m, 1H, Tic–H), 7.58 (s, 1H, Ar–H), 9.42 (broad s, 1H, OH), 12.72 (broad s, 1H, COOH); TOFMS m/z 445 (M^+-Boc).

7-OH-Boc-L-Tic. To a solution of 7-OH-6,8-diiodo-Boc-L-Tic (10.4 g, 19.1 mmol, 1 equiv) in 385 mL of distilled MeOH, were added distilled triethylamine (5.3 mL, 38.2 mmol, 2 equiv) and Pd/C 10% (1.18 g). After stirring the mixture under hydrogene pressure (100 bars) for 4 h, the solution was filtered on Celite, the filtrate concentrated with the residue being taken up in 200 mL of a H_2O/AcOEt 1:1 mixture. The aqueous layer was acidified with concentrated chlorhydric acid 35% until pH 2–3 then extracted with 3×150 mL of AcOEt. The organic layers were mixed, dried over $MgSO_4$, the solvent evaporated and the residue purified by column chromatography (CH_2Cl_2/MeOH, 97:3) to yield 7-OH-Boc-L-Tic as a yellow solid (3.0 mg, 54% yield); R_f 0.65 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 17.42 min; ^{1}H NMR (DMSO-d_6) δ 1.33 + 1.40 (s, 9H, $C(CH_3)_3$ *cis*/*trans*), 2.96 (m, 2H, Tic–H), 4.20–4.48 (m, 2H, Tic–H), 4.50–4.74 (m, 1H, Tic–H), 6.49 (s, 1H, Ar–H), 6.62–6.65 (d, $J=7.7$ Hz, 1H, Ar–H), 6.90 (d, $J=7.9$ Hz, 1H, Ar–H), 9.25 (broad s, 1H, OH); TOFMS m/z 193 (M^+-Boc).

7-OH-Boc-L-Tic-pyrrolidine. 7-OH-Boc-L-Tic-pyrrolidine was prepared from 7-OH-Boc-L-Tic (1 g, 3.4 mmol, 1 equiv) and pyrrolidine (285 μL, 3.4 mmol, 1 equiv) by method B and was obtained after TLC (CH_2Cl_2/MeOH, 90:10) as a yellow solid (735 mg, 62% yield); R_f 0.75 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 17.92 min; ^{1}H NMR (DMSO-d_6) δ 1.33 + 1.42 (s, 9H, $C(CH_3)_3$ *cis*/*trans*), 1.75–1.89 (m, 4H, pyrrolidine), 2.45–2.49 + 2.96–2.99 (m, 2H, Tic–H *cis*/*trans*), 3.29–3.50 (m, 4H, pyrrolidine), 4.14 + 4.59 (d, $J=15.2$ Hz, 2H, Tic–H), 4.42–4.45 + 4.81–4.84 (m, 1H, Tic–H cis/trans), 6.51 (s, 1H, Ar–H), 6.59 (d, $J=7.2$ Hz, 1H, Ar–H), 6.90 (d, $J=7.1$ Hz, 1H, Ar–H), 9.24 (broad s, 1H, OH); TOFMS m/z 346 (M^+).

4-Phenylbutyryl-7-OH-L-Tic-pyrrolidine (4). Compound **4** was prepared from 7-OH-Boc-L-Tic-pyrrolidine (700 mg, 2.0 mmol, 1 equiv) and 4-phenylbutyric acid (335 mg, 2.0 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 95:5) as a colourless oil (300 mg, 38% yield); R_f 0.70 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 19.57 min; ^{1}H NMR (acetone d_6) δ 1.56–1.63 (m, 2H, pyrrolidine), 1.74–1.78 (m, 4H, $CH_2\underline{CH_2}CH_2C_6H_5$ and pyrrolidine), 2.32 (t, $J=7.2$ Hz, 2H, $\underline{CH_2}CH_2CH_2C_6H_5$), 2.46 (t, $J=7.6$ Hz, 2H, $CH_2CH_2\underline{CH_2}C_6H_5$), 2.74–2.86

(m, 2H, Tic–H), 3.08–3.25 (m, 2H, pyrrolidine), 3.35–3.57 (m, 2H, pyrrolidine), 4.37 (d, $J=15.3$ Hz, 1H, Tic–H), 4.47 (d, $J=15.3$ Hz, 1H, Tic–H), 4.61 (t, $J=6.5$ Hz, 1H, Tic–H), 6.52 (s, 1H, Ar–H), 6.53 (d, $J=2.4$ Hz, 1H, Ar–H), 6.80–6.85 (m, 1H, Ar–H), 6.95–7.10 (m, 5H, Ar–H); TOFMS m/z 392 (M^+).

4-Phenylbutyryl-7-OEt-L-Tic-pyrrolidine (5). To a solution of compound **4** (100 mg, 0.26 mmol, 1 equiv) in 5 mL of DMF, were added ethylbromide (29 μL, 0.38 mmol, 1.5 equiv) and K_2CO_3 (70 mg, 0.51 mmol, 2 equiv). After stirring the mixture at 60 °C for 18 h, the solvent was evaporated and the residue taken up in 20 mL of AcOEt, washed by 2×20 mL of a solution of HCl 1 M and brine. The organic layer was then dried over $MgSO_4$ and the residue purified by TLC (CH_2Cl_2/MeOH, 94:6) to yield compound **5** as a colourless oil (70 mg, 67% yield); R_f 0.65 (CH_2Cl_2/MeOH, 9.4:0.6); HPLC t_R 25.12 min; ^{1}H NMR ($CDCl_3$) δ 1.39 (t, $J=7.0$ Hz, 3H, $OCH_2\underline{CH_3}$), 1.80–2.03 (m, 6H, $CH_2\underline{CH_2}CH_2C_6H_5$ and pyrrolidine), 2.42–2.48 (m, 2H, $\underline{CH_2}CH_2CH_2C_6H_5$), 2.68 (t, $J=7.3$ Hz, 2H, $CH_2CH_2\underline{CH_2}C_6H_5$), 2.99 (d, $J=6.6$ Hz, 2H, Tic-H), 3.33–3.52 (m, 3H, pyrrolidine), 3.74–3.80 (m, 1H, pyrrolidine), 3.98 (q, $J=7.0$ Hz, 2H, $O\underline{CH_2}CH_3$), 4.48 (d, $J=15.3$ Hz, 1H, Tic–H), 4.62 (d, $J=15.3$ Hz, 1H, Tic–H), 5.11 (t, $J=6.6$ Hz, 1H, Tic–H), 6.60 (d, $J=2.4$ Hz, 1H, Ar–H), 6.74 (dd, $J=2.4$, 8.3 Hz, 1H, Ar–H), 7.04 (d, $J=8.3$ Hz, 1H, Ar–H), 7.14–7.30 (m, 5H, Ar–H); TOFMS m/z 420 (M^+).

4-Benzoyl-L-Tic-pyrrolidine (6). Compound **6** was prepared from Boc-L-Tic-pyrrolidine (120 mg, 0.52 mmol, 1 equiv) and benzoic acid (65 mg, 0.52 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 95:5) as a colourless oil (105 mg, 69% yield); R_f 0.60 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 19.63 min; ^{1}H NMR ($CDCl_3$) δ 1.80–2.10 (m, 4H, pyrrolidine), 3.10–3.25 (m, 2H, Tic–H), 3.41–3.92 (m, 4H, pyrrolidine), 4.60 (d, $J=15.6$ Hz, 1H, Tic–H), 4.68 (d, $J=15.6$ Hz, 1H, Tic–H), 5.18 (t, $J=7.1$ Hz, 1H, Tic–H), 6.90–7.61 (m, 9H, Ar–H); TOFMS m/z 334 (M^+).

Phenylacetyl-L-Tic-pyrrolidine (7). Compound **7** was prepared from Boc-L-Tic-pyrrolidine (120 mg, 0.52 mmol, 1 equiv) and benzylic acid (70 mg, 0.52 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 95:5) as a colourless oil (120 mg, 67% yield); R_f 0.75 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 20.48 min; ^{1}H NMR ($CDCl_3$) δ 1.80–2.00 (m, 4H, pyrrolidine), 3.07 (d, $J=6.8$ Hz, 2H, Tic–H), 3.30–3.56 (m, 3H, pyrrolidine), 3.72–3.75 (m, 1H, pyrrolidine), 3.85 (s, 2H, CH_2), 4.68 (s, 2H, Tic–H), 5.10 (t, $J=6.8$ Hz, 1H, Tic–H), 6.90–7.30 (m, 9H, Ar–H); TOFMS m/z 348 (M^+).

3-Phenylpropanoyl-L-Tic-pyrrolidine (8). Compound **8** was prepared from Boc-L-Tic-pyrrolidine (120 mg, 0.52 mmol, 1 equiv) and 3-phenylpropanoic acid (80 mg, 0.52 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 95:5) as a colourless oil (145 mg, 76% yield); R_f 0.55 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 22.19 min; ^{1}H NMR ($CDCl_3$) δ 1.70–1.90 (m, 4H, pyrrolidine), 2.60–3.00 (m, 4H, CH_2CH_2), 3.04 (d, $J=6.6$ Hz, 2H, Tic–H), 3.30–3.51 (m, 3H, pyrrolidine), 3.70–3.73 (m, 1H, pyrrolidine), 4.57 (d, $J=15.3$ Hz, 1H, Tic–H), 4.64 (d, $J=15.3$ Hz, 1H, Tic–H), 5.12 (t, $J=6.6$ Hz, 1H, Tic–H), 7.00–7.27 (m, 9H, Ar–H); TOFMS m/z 362 (M^+).

5-Phenylpentanoyl-L-Tic-pyrrolidine (9). Compound **9** was prepared from Boc-L-Tic-pyrrolidine (90 mg, 0.40 mmol, 1 equiv) and 5-phenylpentanoic acid (70 mg, 0.40 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 96:4) as a colourless oil (100 mg, 63% yield); R_f 0.80 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 24.48 min; ^{1}H NMR ($CDCl_3$) δ 1.69 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$), 1.80–2.06 (m, 4H, pyrrolidine), 2.50–2.64 (m, 4H, $\underline{CH_2}CH_2CH_2\underline{CH_2}$), 3.07 (d, $J=6.6$ Hz, 2H, Tic–H), 3.30–3.52 (m, 3H, pyrrolidine), 3.75–3.82 (m, 1H, pyrrolidine), 4.62 (d, $J=15.3$ Hz, 1H, Tic–H), 4.69 (d, $J=15.3$ Hz, 1H, Tic–H), 5.14 (t, $J=6.6$ Hz, 1H, Tic–H), 7.07–7.29 (m, 9H, Ar–H); TOFMS m/z 390 (M^+).

4-(4-Nitrophenyl)butyryl-L-Tic-pyrrolidine (10). Compound **10** was prepared from Boc-L-Tic-pyrrolidine (110 mg, 0.48 mmol, 1 equiv) and 4-(4-nitrophenyl)butyric acid (100 mg, 0.48 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 95:5) as a colourless oil (115 mg, 58% yield); R_f 0.95 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 22.88 min; ^{1}H NMR (acetone-d_6) δ 1.59–1.86 (m, 6H, $CH_2\underline{CH_2}CH_2C_6H_5$ and pyrrolidine), 2.42 (t, $J=7.4$ Hz, 2H, $\underline{CH_2}CH_2CH_2C_6H_4NO_2$), 2.69 (t, $J=7.7$ Hz, 2H, $CH_2\underline{CH_2}\underline{CH_2}C_6H_5NO_2$), 2.84–2.99 (m, 2H, Tic–H), 3.05–3.58 (m, 4H, pyrrolidine), 4.47 (d, $J=15.8$ Hz, 1H, Tic–H), 4.65 (d, $J=15.8$ Hz, 1H, Tic–H), 5.03 (t, $J=6.2$ Hz, 1H, Tic–H), 7.00–7.09 (m, 4H, Ar–H), 7.37 (d, $J=8.7$ Hz, 2H, Ar–H), 8.00 (d, $J=8.7$ Hz, 2H, Ar–H); TOFMS m/z 421 (M^+).

4-(4-Methoxyphenyl)butyryl-L-Tic-pyrrolidine (11). Compound **11** was prepared from Boc-L-Tic-pyrrolidine (120 mg, 0.52 mmol, 1 equiv) and 4-(4-methoxyphenyl)butyric acid (100 mg, 0.52 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 95:5) as a colourless oil (145 mg, 70% yield); R_f 0.75 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 22.75 min; ^{1}H NMR (acetone-d_6) δ 1.53–1.77 (m, 6H, $CH_2\underline{CH_2}CH_2C_6H_5$ and pyrrolidine), 2.33 (t, $J=7.3$ Hz, 2H, $\underline{CH_2}CH_2CH_2C_6H_4OMe$), 2.44 (t, $J=7.6$ Hz, 2H, $CH_2\underline{CH_2}\underline{CH_2}C_6H_5OMe$), 2.92 (d, $J=5.8$ Hz, 2H, Tic–H), 3.00–3.62 (m, 4H, pyrrolidine), 3.59 (s, 3H, OCH_3), 4.45 (d, $J=15.8$ Hz, 1H, Tic–H), 4.59 (d, $J=15.8$ Hz, 1H, Tic–H), 5.04 (t, $J=6.1$ Hz, 1H, Tic–H), 6.68 (d, $J=8.5$ Hz, 2H, Ar–H), 6.93 (d, $J=8.5$ Hz, 2H, Ar–H), 6.99–7.06 (m, 4H, Ar–H); TOFMS m/z 406 (M^+).

4-(Thiophen-2-yl)butyryl-L-Tic-pyrrolidine (12). Compound **12** was prepared from Boc-L-Tic-pyrrolidine (110 mg, 0.48 mmol, 1 equiv) and 4-(thiophen-2-yl)butyric acid (80 mg, 0.48 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 98:2) as a yellow oil (75 mg, 40% yield); R_f 0.85 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 22.66 min; ^{1}H NMR (acetone-d_6) δ 1.57–1.92 (m, 6H, $COCH_2\underline{CH_2}CH_2$ and pyrrolidine), 2.38 (t, $J=7.3$ Hz, 2H, $CO\underline{CH_2}CH_2CH_2$), 2.72 (t, $J=6.2$ Hz, 2H, $COCH_2CH_2\underline{CH_2}$), 2.88–2.98 (m, 2H, Tic–H), 3.03–

3.75 (m, 4H, pyrrolidine), 4.46 (d, $J=15.5$ Hz, 1H, Tic–H), 4.62 (d, $J=15.5$ Hz, 1H, Tic–H), 5.04 (t, $J=6.2$ Hz, 1H, Tic–H), 6.65–6.72 (m, 1H, Ar–H), 6.72–6.79 (m, 1H, Ar–H), 6.98–7.08 (m, 5H, Ar–H); TOFMS m/z 382 (M^+).

4-(Pyren-1-yl)butyryl-L-Tic-pyrrolidine (13). Compound **13** was prepared from Boc-L-Tic-pyrrolidine (140 mg, 0.61 mmol, 1 equiv) and 4-(pyren-1-yl)butyric acid (175 mg, 0.61 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 96:4) as a yellow oil (200 mg, 66% yield); R_f 0.60 (CH_2Cl_2/MeOH, 9.6:0.4); HPLC t_R 32.19 min; ^{1}H NMR ($CDCl_3$) δ 1.82–2.03 (m, 4H, pyrrolidine), 2.17–2.30 (m, 2H, $COCH_2\underline{CH_2}CH_2$), 2.54–2.61 (m, 2H, $CO\underline{CH_2}CH_2CH_2$), 3.07 (d, $J=6.6$ Hz, 2H, Tic–H), 3.38–3.55 (m, 5H, $COCH_2CH_2\underline{CH_2}$ and pyrrolidine), 3.75–3.80 (m, 1H, pyrrolidine), 4.48 (d, $J=15.3$ Hz, 1H, Tic–H), 4.62 (d, $J=15.3$ Hz, 1H, Tic–H), 5.16 (t, $J=6.6$ Hz, 1H, Tic–H), 6.90 (d, $J=7.4$ Hz, 1H, Ar–H), 7.10–7.20 (m, 3H, Ar–H), 7.87 (d, $J=7.8$ Hz, 1H, Ar–H), 7.98–8.10 (m, 5H, Ar–H), 8.16 (d, $J=7.5$ Hz, 2H, Ar–H), 8.31 (d, $J=9.3$ Hz, 1H, Ar–H); TOFMS m/z 500 (M^+).

Pyrid-1-ylacetyl-L-Tic-pyrrolidine (14). Compound **14** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and 4-(pyrid-3-yl)acetic acid, HCl (175 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 90:10) as a yellow solid (115 mg, 37% yield); R_f 0.20 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 13.27 min; ^{1}H NMR ($CDCl_3$) δ 1.83–2.01 (m, 4H, pyrrolidine), 3.08–3.12 (m, 2H, Tic–H), 3.40–3.52 (m, 3H, pyrrolidine), 3.78–3.86 (m, 1H, pyrrolidine), 3.87 (s, 2H, CH_2), 4.62 (d, $J=14.9$ Hz, 1H, Tic–H), 4.75 (d, $J=14.9$ Hz, 1H, Tic–H), 5.06 (t, $J=7.0$ Hz, 1H, Tic–H), 6.99 (d, $J=6.9$ Hz, 1H, Ar–H), 7.16–7.24 (m, 5H, Ar–H), 8.53 (d, $J=4.3$ Hz, 2H, Ar–H); TOFMS m/z 349 (M^+).

Pyrid-1-ylcarbonyl-L-Tic-pyrrolidine (15). Compound **15** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and isonicotinic acid (125 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 93:7) as a yellow solid (185 mg, 60% yield); R_f 0.20 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 13.64 min; ^{1}H NMR ($CDCl_3$) δ 1.88–2.05 (m, 4H, pyrrolidine), 3.19 (d, $J=7.3$ Hz, 2H, Tic–H), 3.42–3.63 (m, 3H, pyrrolidine), 3.86–3.93 (m, 1H, pyrrolidine), 4.43 (d, $J=15.1$ Hz, 1H, Tic–H), 4.71 (d, $J=15.1$ Hz, 1H, Tic–H), 5.09 (t, $J=7.3$ Hz, 1H, Tic–H), 6.95 (d, $J=7.3$ Hz, 1H, Ar–H), 7.19–7.27 (m, 3H, Ar–H), 7.35 (dd, $J=1.6$, 4.4 Hz, 2H, Ar–H), 8.73 (dd, $J=1.6$, 4.4 Hz, 2H, Ar–H); TOFMS m/z 335 (M^+).

4-Methylcyclohexylacetyl-L-Tic-pyrrolidine (16). Compound **16** was prepared from Boc-L-Tic-pyrrolidine (90 mg, 0.40 mmol, 1 equiv) and 4-methylcyclohexylacetic acid (60 mg, 0.40 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 97:3) as a colourless oil (90 mg, 61% yield); R_f 0.70 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 25.29 min; ^{1}H NMR ($CDCl_3$) δ 0.77 + 0.84 (d, $J=6.8$ Hz, 3H, CH_3 *cis*/*trans*), 1.18–1.96 (m, 14H, cyclohexyle and pyrrolidine), 2.26 + 2.36 (d, $J=7.0$ Hz, 2H, $COCH_2$ *cis*/*trans*), 3.00 (d, $J=6.6$ Hz, 2H, Tic–H), 3.27–3.47 (m, 3H, pyrrolidine), 3.68–3.72 (m, 1H, pyrrolidine), 4.62–4.65 (m, 2H, Tic–H), 5.06 (t, $J=6.6$ Hz, 1H, Tic–H), 7.03–7.20 (m, 4H, Ar–H); TOFMS m/z 368 (M^+).

4-Methylcyclohexylcarbonyl-L-Tic-pyrrolidine (17). Compound **17** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and 4-methylcyclohexylcarboxylic acid (140 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 93:7) as a yellow oil (295 mg, 92% yield); R_f 0.45 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 23.38 min; ^{1}H NMR ($CDCl_3$) δ 0.90 + 0.97 (d, $J=7.0$ Hz, 3H, CH_3 *cis*/*trans*), 1.50–1.99 (m, 13H, cyclohexyle and pyrrolidine), 2.50–2.69 (m, 1H, cyclohexyle), 3.03–3.11 (m, 2H, Tic–H), 3.37–3.53 (m, 3H, pyrrolidine), 3.69–3.75 (m, 1H, pyrrolidine), 4.65–4.72 (m, 2H, Tic–H), 5.11 (dd, $J=6.7$, 15.2 Hz, 1H, Tic–H), 7.13–7.24 (m, 4H, Ar–H); TOFMS m/z 354 (M^+).

Cyclohexylcarbonyl-L-Tic-pyrrolidine (18). Compound **18** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and cyclohexylcarboxylic acid (130 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 93:7) as a colourless oil (135 mg, 44% yield); R_f 0.40 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 21.63 min; ^{1}H NMR ($CDCl_3$) δ 1.25–2.03 (m, 13H, cyclohexyle and pyrrolidine), 2.59–2.67 (m, 1H, cyclohexyle), 3.03 (dd, $J=6.6$, 15.4 Hz, 1H, Tic–H), 3.12 (dd, $J=6.6$, 15.4 Hz, 1H, Tic–H), 3.37–3.53 (m, 3H, pyrrolidine), 3.70–3.75 (m, 1H, pyrrolidine), 4.68 (d, $J=15.3$ Hz, 1H, Tic–H), 4.74 (d, $J=15.3$ Hz, 1H, Tic–H), 5.13 (t, $J=6.6$ Hz, 1H, Tic–H), 7.13–7.24 (m, 4H, Ar–H); TOFMS m/z 340 (M^+).

Cyclohexylacetyl-L-Tic-pyrrolidine (19). Compound **19** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and cyclohexylacetic acid (140 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 93:7) as a colourless oil (120 mg, 37% yield); R_f 0.40 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 23.51 min; ^{1}H NMR ($CDCl_3$) δ 0.82–2.07 (m, 15H, cyclohexyle and pyrrolidine), 2.38 (d, $J=6.6$ Hz, 2H, $COCH_2$), 3.04 (dd, $J=6.7$, 15.3 Hz, 1H, Tic–H), 3.12 (dd, $J=6.8$, 15.4 Hz, 1H, Tic–H), 3.37–3.51 (m, 3H, pyrrolidine), 3.70–3.72 (m, 1H, pyrrolidine), 4.67 (d, $J=15.3$ Hz, 1H, Tic–H), 4.74 (d, $J=15.3$ Hz, 1H, Tic–H), 5.13 (t, $J=6.5$ Hz, 1H, Tic–H), 7.15–7.26 (m, 4H, Ar–H); TOFMS m/z 354 (M^+).

3-Cyclohexylpropanoyl-L-Tic-pyrrolidine (20). Compound **20** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and 3-cyclohexylpropanoic acid (155 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 93:7) as a colourless oil (95 mg, 29% yield); R_f 0.40 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 25.32 min; ^{1}H NMR ($CDCl_3$) δ 0.90–1.00 (m, 2H, cyclohexyle), 1.18–1.28 (m, 5H, cyclohexyle), 1.50–1.55 (m, 2H, $COCH_2\underline{CH_2}$), 1.66–1.74 (m, 4H, cyclohexyle), 1.82–2.04 (m, 4H, pyrrolidine), 2.43–2.49 (m, 2H, $CO\underline{CH_2}CH_2$), 3.07 (d, $J=6.6$ Hz, 2H, Tic–H), 3.35–3.53 (m, 3H, pyrrolidine), 3.75–3.80 (m, 1H, pyrrolidine), 4.66 (d, $J=15.3$ Hz, 1H, Tic–H), 4.72 (d,

J = 15.3 Hz, 1H, Tic–H), 5.15 (t, J = 6.6 Hz, 1H, Tic–H), 7.12–7.23 (m, 4H, Ar–H); TOFMS m/z 368 (M^+).

4-Cyclohexylbutyryl-L-Tic-pyrrolidine (21). Compound **21** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and 4-cyclohexylbutyric acid (170 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 93:7) as a yellow oil (280 mg, 81% yield); R_f 0.45 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 27.93 min; ^{1}H NMR ($CDCl_3$) δ 0.78–0.96 (m, 2H, cyclohexyle), 1.18–1.26 (m, 6H, $COCH_2CH_2\underline{CH_2}$ and cyclohexyle), 1.65–2.04 (m, 11H, $COCH_2\underline{CH_2}\underline{CH_2}$ and cyclohexyle and pyrrolidine), 2.42–2.46 (m, 2H, $CO\underline{CH_2}CH_2CH_2$), 3.07 (d, J = 6.6 Hz, 2H, Tic–H), 3.36–3.54 (m, 3H, pyrrolidine), 3.75–3.83 (m, 1H, pyrrolidine), 4.66 (d, J = 15.3 Hz, 1H, Tic–H), 4.72 (d, J = 15.3 Hz, 1H, Tic–H), 5.15 (t, J = 6.6 Hz, 1H, Tic–H), 7.12–7.23 (m, 4H, Ar–H); TOFMS m/z 382 (M^+).

5-Cyclohexylpentanoyl-L-Tic-pyrrolidine (22). Compound **22** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and 5-cyclohexylpentanoic acid (185 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 93:7) as a yellow oil (180 mg, 50% yield); R_f 0.40 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 29.90 min; ^{1}H NMR ($CDCl_3$) δ 0.79–0.88 (m, 2H, cyclohexyle), 1.16–1.37 (m, 6H, $COCH_2CH_2\underline{CH_2}CH_2$ and cyclohexyle), 1.56–1.69 (m, 8H, $COCH_2\underline{CH_2}CH_2CH_2$ and cyclohexyle), 1.83–2.02 (m, 5H, cyclohexyle and pyrrolidine), 2.42–2.46 (m, 2H, $CO\underline{CH_2}CH_2CH_2$), 3.08 (d, J = 6.6 Hz, 2H, Tic–H), 3.35–3.54 (m, 3H, pyrrolidine), 3.73–3.83 (m, 1H, pyrrolidine), 4.66 (d, J = 15.6 Hz, 1H, Tic–H), 4.72 (d, J = 15.6 Hz, 1H, Tic–H), 5.17 (t, J = 6.6 Hz, 1H, Tic–H), 7.12–7.26 (m, 4H, Ar–H); TOFMS m/z 396 (M^+).

Cyclopentylacetyl-L-Tic-pyrrolidine (23). Compound **23** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and cyclopentylacetic acid (130 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 93:7) as a colourless oil (200 mg, 65% yield); R_f 0.50 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 22.09 min; ^{1}H NMR ($CDCl_3$) δ 1.06–1.62 (m, 8H, cyclopentyle), 1.82–2.02 (m, 4H, pyrrolidine), 2.25–2.36 (m, 1H, cyclopentyle), 2.44 (d, J = 7.1 Hz, 2H, $COCH_2$), 3.07 (d, J = 6.4 Hz, 2H, Tic–H), 3.33–3.52 (m, 3H, pyrrolidine), 3.73–3.82 (m, 1H, pyrrolidine), 4.70 (s, 2H, Tic–H), 5.19 (t, J = 6.4 Hz, 1H, Tic–H), 7.12–7.26 (m, 4H, Ar–H); TOFMS m/z 340 (M^+).

Norborn-2-ylacetyl-L-Tic-pyrrolidine (24). Compound **24** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and norborn-2-ylacetic acid (155 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 93:7) as a colourless oil (100 mg, 30% yield); R_f 0.40 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 24.25 min; ^{1}H NMR ($CDCl_3$) δ 1.10–2.43 (m, 17H, $COCH_2$ and norbornyl and pyrrolidine), 3.07 (d, J = 6.3 Hz, 2H, Tic–H), 3.35–3.54 (m, 3H, pyrrolidine), 3.73–3.79 (m, 1H, pyrrolidine), 4.69 (s, 2H, Tic–H), 5.22 (t, J = 6.3 Hz, 1H, Tic–H), 7.12–7.23 (m, 4H, Ar–H); TOFMS m/z 366 (M^+).

Adamant-1-ylacetyl-L-Tic-pyrrolidine (25). Compound **25** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and adamant-1-ylacetic acid (195 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 93:7) as a colourless oil (145 mg, 39% yield); R_f 0.35 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 27.58 min; ^{1}H NMR ($CDCl_3$) δ 1.61–1.99 (m, 19H, adamantyl and pyrrolidine), 2.30 (d, J = 7.1 Hz, 2H, $COCH_2$), 3.07 (d, J = 6.7 Hz, 2H, Tic–H), 3.36–3.53 (m, 3H, pyrrolidine), 3.75–3.81 (m, 1H, pyrrolidine), 4.68 (d, J = 15.2 Hz, 1H, Tic–H), 4.80 (d, J = 15.2 Hz, 1H, Tic–H), 5.15 (t, J = 6.7 Hz, 1H, Tic–H), 7.11–7.22 (m, 4H, Ar–H); TOFMS m/z 406 (M^+).

Dicyclohexylacetyl-L-Tic-pyrrolidine (26). Compound **26** was prepared from Boc-L-Tic-pyrrolidine (210 mg, 0.91 mmol, 1 equiv) and dicyclohexylacetic acid (225 mg, 1 mmol, 1.1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 93:7) as a colourless oil (185 mg, 46% yield); R_f 0.55 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 20.05 min; ^{1}H NMR ($CDCl_3$) δ 0.98–1.27 (m, 10H, cyclohexyle), 1.66–1.74 (m, 12H, cyclohexyle), 1.86–1.94 (m, 2H, pyrrolidine), 1.99–2.09 (m, 3H, COCH and pyrrolidine), 3.12–3.20 (m, 2H, Tic–H), 3.41–3.60 (m, 3H, pyrrolidine), 3.76–3.84(m, 1H, pyrrolidine), 4.74 (d, J = 15.2 Hz, 1H, Tic–H), 4.82–4.89 (m, 2H, Tic-H), 7.17–7.30 (m, 4H, Ar–H); TOFMS m/z 436 (M^+).

Boc-L-Pro-OMe. To a solution of Boc-L-Pro (1 g, 4.7 mmol, 1 equiv) in 20 mL of MeOH, was added 1.3 mL of a solution of Cs_2CO_3 1M (2.3 mmol, 0.5 equiv). The solvent was evaporated and the residue taken up in 3×30 mL of a toluene/EtOH 1:1 mixture and concentrated. To a solution of the cesium salt in 10 mL of DMF was added CH_3I (725 mg, 5.1 mmol, 1.1 equiv). After stirring the mixture at room temperature for 2 h, the solvent was evaporated with the residue being taken up in 20 mL of AcOEt and washed with 20 mL of brine. The organic layer was separated, dried over $MgSO_4$ and the solvent evaporated to yield Boc-L-Pro-OMe as a colourless oil (985 mg, 93% yield); R_f 0.75 (CH_2Cl_2/MeOH, 9.4:0.6); HPLC t_R 18.15 min; ^{1}H NMR ($CDCl_3$) δ 1.41 + 1.46 (s, 9H, $C(CH_3)_3$ *cis*/*trans*), 1.83–2.27 (m, 4H, CH_2), 3.37–3.60 (m, 2H, CH_2), 3.72 (s, 3H, OCH_3), 4.20–4.24 + 4.30–4.34 (m, 1H, CH *cis*/*trans*); TOFMS m/z 229 (M^+).

Boc-L-Tic-L-Pro-OMe. Boc-L-Tic-L-Pro-OMe was prepared from Boc-L-Pro-OMe (1 g, 4.4 mmol, 1 equiv) and Boc-L-Tic (1.21 g, 4.4 mmol, 1 equiv) by method C and was obtained after column chromatography (CH_2Cl_2/MeOH, 95:5) as a colourless oil (1.29 g, 75% yield); R_f 0.50 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 21.39 min; ^{1}H NMR ($CDCl_3$) δ 1.42 + 1.48 (s, 9H, $C(CH_3)_3$ *cis*/*trans*), 1.94–2.18 (m, 4H, pyrrolidine), 3.04–3.14 (m, 2H, Tic–H), 3.60 + 3.69 (s, 3H, OCH_3 *cis*/*trans*), 3.65–3.77 (m, 2H, pyrrolidine), 4.31 + 4.48 (d, J = 15.5 + 15.9 Hz, 1H, Tic–H *cis*/*trans*), 4.51–4.55 (m, 1H, pyrrolidine), 4.61 + 5.02 (t, J = 7.0 + 5.9 Hz, 1H, Tic–H), 4.80 + 4.96 (d, J = 15.5 + 15.9 Hz, 1H, Tic–H *cis*/*trans*), 7.11–7.26 (m, 4H, Ar–H); TOFMS m/z 388 (M^+).

4-Phenylbutyryl-L-Tic-L-Pro-OMe. 4-phenylbutyryl-L-Tic-L-Pro-OMe was prepared from Boc-L-Tic-L-Pro-OMe (425 mg, 1.1 mmol, 1 equiv) and 4-phenylbutyric acid (180 mg, 1.1 mmol, 1 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 94:6) as a colourless oil (252 mg, 54% yield); R_f 0.40 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 22.97 min; ^1H NMR ($CDCl_3$) δ 1.93–2.20 (m, 6H, $COCH_2\underline{CH_2}CH_2$ and pyrrolidine), 2.42 (t, $J=7.4$ Hz, 2H, $CO\underline{CH_2}CH_2CH_2$), 2.65 (t, $J=7.4$ Hz, 2H, $COCH_2CH_2\underline{CH_2}$), 3.07–3.11 (m, 2H, Tic–H), 3.62 (s, 3H, OCH_3), 3.64–3.71 (m, 2H, pyrrolidine), 4.47–4.60 (m, 3H, Tic–H and pyrrolidine), 4.99 (t, $J=7.3$ Hz, 1H, Tic–H), 7.02–7.26 (m, 9H, Ar–H); TOFMS m/z 434 (M^+).

4-Phenylbutyryl-L-Tic-Pro-CHO (27). To a solution of 4-phenylbutyryl-L-Tic-L-Pro-OMe (200 mg, 0.46 mmol, 1 equiv) in 5 mL of CH_2Cl_2 dried over molecular sieves, was added at −70 °C, a solution of DIBAL 1 M in CH_2Cl_2 (1.38 mL, 1.38 mmol, 3 equiv). After stirring the mixture at −70 °C for 2 h, 2 mL of isopropanol was added, the solution stirred for a further 10 min, warmed to room temperature, then washed with 2×10 mL of water. The organic layer was dried over $MgSO_4$, the solvent evaporated and the residue purified by column chromatography (CH_2Cl_2/MeOH/pyridine, 90:10:0.1) to yield **27** as a colourless oil under the racemic form (30 mg, 16% yield); R_f 0.50 (AcOEt/cyclohexane, 5:5); HPLC t_R 21.17 min (broad); ^1H NMR ($CDCl_3$) δ 1.84–2.06 (m, 6H, $COCH_2\underline{CH_2}CH_2$ and pyrrolidine), 2.41–2.48 (m, 2H, $CO\underline{CH_2}CH_2CH_2$), 2.63–2.70 (m, 2H, $COCH_2CH_2\underline{CH_2}$), 3.00–3.20 (m, 2H, Tic–H), 3.71–3.87 (m, 2H, pyrrolidine), 4.47–4.59 (m, 3H, Tic–H and pyrrolidine), 5.10 (t, $J=7.0$ Hz, 1H, Tic–H), 7.03–7.31 (m, 9H, Ar–H), 9.42 + 9.44 (d + s, 0.7H, $J=2.0$ Hz, CHO), 9.74 + 9.75 (d + s, 0.3H, $J=2.0$ Hz, CHO); TOFMS m/z 404 (M^+).

4-Phenylbutyryl-L-Tic-L-Pro-OCH_2Cl (28). A solution of LDA (5 mmol) was prepared from a solution of diisopropylamine (770 μL, 5.5 mmol, 5.5 equiv) in 5 mL of THF and a solution of n-BuLi 2.5 M (2 mL, 5 mmol, 5 equiv) in hexane. To a solution of 4-phenylbutyryl-L-Tic-L-Pro-OMe (435 mg, 1 mmol, 1 equiv) and chloroiodoethane (291 μL, 4 mmol, 4 equiv) in 5 mL of THF was added, at −70 °C, the solution of LDA (5 mmol) prepared previously. After stirring the mixture at −70 °C for 10 min, 10 mL of a solution of acetic acid 10% in THF was added dropwise within 10 min. The solution was diluted at room temperature in 75 mL of AcOEt, washed with 75 mL of brine, 75 mL of an aqueous solution of $NaHCO_3$ 1 M then 75 mL of brine. The organic layer was dried over $MgSO_4$, the solvent evaporated and the residue purified by column chromatography (AcOEt/cyclohexane, 8:2) to yield compound **28** as a colourless oil (215 mg, 48% yield); R_f 0.40 (AcOEt/cyclohexane, 8:2); HPLC t_R 23.72 min; ^1H NMR ($CDCl_3$) δ 1.91–2.27 (m, 6H, $COCH_2\underline{CH_2}CH_2$ and pyrrolidine), 2.50 (t, $J=7.4$ Hz, 2H, $CO\underline{CH_2}CH_2CH_2$), 2.68 (t, $J=7.4$ Hz, 2H, $COCH_2CH_2\underline{CH_2}$), 3.02–3.16 (m, 2H, Tic–H), 3.63–3.81 (m, 2H, pyrrolidine), 4.15 (s, 2H, $COCH_2Cl$), 4.46–4.63 (m, 3H, Tic–H and pyrrolidine), 4.76–4.82 (m, 1H, Tic–H), 7.06–7.31 (m, 9H, Ar–H); TOFMS m/z 452.5 (M^+).

4-Phenylbutyryl-L-Tic-L-Pro-OCH_2OCOMe (29). To a solution of compound **28** (100 mg, 0.22 mmol, 1 equiv) in 5 mL of DMF, was added potassium acetate (21 mg, 0.22 mmol, 1 equiv). After stirring the mixture at 60 °C for 3 h, the solvent was evaporated with the residue being taken up in 20 mL of a AcOEt/brine 1:1 mixture. The organic layer was dried over $MgSO_4$ and the solvent evaporated to yield compound **29** as a colourless oil (45 mg, 43% yield); R_f 0.25 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 22.92 min; ^1H NMR ($CDCl_3$) δ 1.90–2.25 (m, 9H, $COCH_2\underline{CH_2}CH_2$ and pyrrolidine and $COCH_3$), 2.53 (t, $J=7.4$ Hz, 2H, $CO\underline{CH_2}CH_2CH_2$), 2.72 (t, $J=7.4$ Hz, 2H, $COCH_2CH_2\underline{CH_2}$), 3.00–3.15 (m, 2H, Tic–H), 3.63–3.87 (m, 2H, pyrrolidine), 4.42–4.62 (m, 3H, Tic–H and pyrrolidine), 4.76–4.91 (m, 3H, Tic–H and $COCH_2OAc$), 7.02–7.31 (m, 9H, Ar–H); TOFMS m/z 476 (M^+).

4-Phenylbutyryl-L-Tic-L-Pro-OCH_2OH (30). To a solution of compound **29** (95 mg, 0.20 mmol, 1 equiv) in 5 mL of MeOH, was added Na_2CO_3 (23 mg, 0.22 mmol, 1.1 equiv). After stirring the mixture at room temperature for 30 min, the solvent was evaporated with the residue being taken up in 15 mL of CH_2Cl_2, washed with 2×15 mL of brine. The organic layer was dried over $MgSO_4$, the solvent evaporated and the residue purified by TLC (CH_2Cl_2/MeOH, 92:8) to yield compound **30** as a colourless oil (25 mg, 31% yield); R_f 0.20 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 20.40 min; ^1H NMR ($CDCl_3$) δ 1.87–2.23 (m, 6H, $COCH_2\underline{CH_2}CH_2$ and pyrrolidine), 2.55 (t, $J=7.4$ Hz, 2H, $CO\underline{CH_2}CH_2CH_2$), 2.70 (t, $J=7.4$ Hz, 2H, $COCH_2CH_2\underline{CH_2}$), 3.01–3.16 (m, H, Tic-H), 3.25 (broad s, 1H, OH), 3.63–3.91 (m, H, pyrrolidine), 4.25 + 439 (d, $J=18.9$ Hz, 2H, $\underline{CH_2}OH$ cis/trans), 4.46–4.63 (m, 3H, Tic–H and pyrrolidine), 4.74–4.80 (m, 1H, Tic–H), 7.06–7.31 (m, 9H, Ar–H); TOFMS m/z 434 (M^+).

Boc-L-Pro-NH_2. To a solution of Boc-L-Pro (2.15 g, 10 mmol, 1 equiv), pyridine (500 μL) and Boc_2O (3 g, 13 mmol, 1.3 equiv) in 50 mL of dioxane was added amnonium carbonate (1 g, 13 mmol, 1.3 equiv). After stirring the mixture at room temperature for 18 h, the solvent was evaporated and the residue taken up in 50 mL of AcOEt, washed with 50 mL of an aqueous solution of citric acid 20% and 50 mL of brine. The aqueous layers were mixed and extracted by 250 mL of AcOEt. The organic layers were mixed then dried over $MgSO_4$ and the solvent evaporated to yield Boc-L-Pro-NH_2 as a colourless oil (2.14 g, 99% yield); R_f 0.75 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 11.89 min; ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H, $C(CH_3)_3$), 1.84–2.21 (m, 4H, pyrrolidine), 3.20–3.48 (m, 2H, pyrrolidine), 4.20–4.30 (m, 1H, pyrrolidine), 6.60 + 6.84 (broad s, 2H, NH_2 *cis*/*trans* 50:50); TOFMS m/z 214 (M^+).

4-Phenylbutyryl-OSu. To a solution of 4-phenylbutyric acid (2 g, 12.2 mmol, 1 equiv) in 100 mL of CH_2Cl_2, were added *N*-OHSu (1.4 g, 12.2 mmol, 1 equiv) and DCC (2.5 g, 12.2 mmol, 1 equiv). After stirring the mixture at room temperature for 4 h, the solution was filtered and the filtrate washed with 2×60 mL of an aqueous solution of $NaHCO_3$ 5% and 2×60 mL of

brine. The organic layer was then dried over $MgSO_4$ and the solvent evaporated to yield 4-phenylbutyryl-Osu as a white solid (3 g, 95% yield); R_f 0.90 (CH_2Cl_2/MeOH, 9.5:0.5); HPLC t_R 21.35 min; ^1H NMR ($CDCl_3$) δ 2.04 (qt, $J=7.4$ Hz, 2H, $CH_2\underline{CH_2}CH_2C_6H_5$), 2.58 (t, $J=7.4$ Hz, 2H, $\underline{CH_2}CH_2CH_2C_6H_5$), 2.71 (t, $J=7.4$ Hz, 2H, $CH_2CH_2\underline{CH_2}C_6H_5$), 2.80 (s, 4H, succinimide); TOFMS m/z 261 (M^+).

4-Phenylbutyryl-L-Tic. To a solution of L-Tic (780 mg, 4.4 mmol, 1 equiv) in 10 mL of dioxane, were added 8.8 mL of NaOH 0.5 M and 4-phenylbutyryl-OSu (1.15 g, 4.4 mmol, 1 equiv). After stirring the mixture at room temperature for 18 h, the solvent was evaporated and the residue taken up in 40 mL of water, acidified by concentrated chlorhydric acid until pH 3. The solution was extracted with 3×40 mL of AcOEt, the organic layer dried over $MgSO_4$, the solvent evaporated and the residue purified by TLC (CH_2Cl_2/MeOH, 90:10) to yield 4-phenylbutyryl-L-Tic as a colourless oil (755 mg, 53% yield); R_f 0.70 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 21.26 min; ^1H NMR ($CDCl_3$) δ 1.78–2.07 (m, 2H, $CH_2\underline{CH_2}CH_2C_6H_5$), 2.30–2.43 (m, 2H, $\underline{CH_2}CH_2CH_2C_6H_5$), 2.58–2.66 (m, 2H, $CH_2CH_2\underline{CH_2}C_6H_5$), 2.95–3.24 (m, 2H, H–Tic), 4.50 (s, 2H, H–Tic), 5.30 (m, 1H, H–Tic), 7.02–7.29 (m, 4H, Ar–H); TOFMS m/z 323 (M^+).

4-Phenylbutyryl-L-Tic-L-Pro-NH_2. 4-phenylbutyryl-L-Tic-L-Pro-NH_2 was prepared from Boc-L-Pro-NH_2 (275 mg, 1.3 mmol, 1 equiv) and 4-phenylbutyryl-L-Tic (415 mg, 3.9 mmol, 3 equiv) by method C and was obtained after TLC (CH_2Cl_2/MeOH, 95:5) as a colourless oil (150 mg, 28% yield); R_f 0.75 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 18.00 min; ^1H NMR ($CDCl_3$) δ 1.82–2.07 (m, 2H, $CH_2\underline{CH_2}CH_2C_6H_5$), 2.18–2.44 (m, 6H, $\underline{CH_2}CH_2CH_2C_6H_5$ and pyrrolidine), 2.59–2.64 (m, 2H, $CH_2CH_2\underline{CH_2}C_6H_5$), 2.95–3.18 (m, 2H, H–Tic), 3.58–3.74 (m, 2H, pyrrolidine), 4.38–4.93 (m, 4H, H–Tic and pyrrolidine), 6.61 + 6.82 (broad s, 2H, NH_2), 7.05–7.25 (m, 9H, Ar–H); TOFMS m/z 373 (M^+).

4-Phenylbutyryl-L-Tic-L-Pro-CN (31). To a solution of 4-phenylbutyryl-L-Tic-L-Pro-NH_2 (100 mg, 0.24 mmol, 1 equiv) in 5 mL of THF was added trifluoroacetic anhydride (67 μL, 0.48 mmol, 2 equiv). After stirring the mixture at room temperature for 3 h, the solvent was evaporated and the residue purified by TLC (CH_2Cl_2/MeOH, 96:4) to yield **31** as a colourless oil (80 mg, 84% yield); R_f 0.80 (CH_2Cl_2/MeOH, 9:1); HPLC t_R 22.51 min; ^1H NMR ($CDCl_3$) δ 1.92–2.04 (m, 2H, $CH_2\underline{CH_2}CH_2C_6H_5$), 2.18–2.25 (m, 4H, pyrrolidine), 2.44–2.51 (m, 2H, $\underline{CH_2}CH_2CH_2C_6H_5$), 2.64–2.69 (m, 2H, $CH_2CH_2\underline{CH_2}C_6H_5$), 3.12 (d, $J=5.6$ Hz, 2H, H-Tic), 3.67–3.88 (m, 2H, pyrrolidine), 4.48 (d, $J=14.5$ Hz, 1H, H–Tic), 4.60 (d, $J=14.5$ Hz, 1H, H–Tic), 4.72 (m, 1H, Tic–H), 4.85 (m, 1H, pyrrolidine), 7.08–7.32 (m, 9H, Ar–H); TOFMS m/z 401 (M^+).

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