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European Journal of Medicinal Chemistry 43 (2008) 2699–2716
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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Original article

Tryptophan-containing dipeptide derivatives as potent PPARγ antagonists: Design, synthesis, biological evaluation, and molecular modeling

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Received 20 August 2007; received in revised form 15 January 2008; accepted 18 January 2008
Available online 3 February 2008

Abstract

The discovery of peroxisome proliferator-activated receptor γ (PPARγ) antagonists (also termed ''selective PPARγ modulators, SPPARγM'') is now of a great interest in the treatment of diabetes and obesity. The structure of compound **1a** (G3335, Fig. 1), a novel class of PPARγ antagonist, is entirely different from that of other reported PPARγ antagonists. A series of 35 novel analogues (**1b–l**, **9a–d**, **13a–t**) were designed, synthesized and evaluated against the agonistic effects exerted by rosiglitazone. These results indicated that most functional groups of **1a** were conserved, and six new compounds (**1b**, **1c**, and **9a–d**) exhibited strong PPARγ antagonistic activities (IC_{50} values of 5.2–25.8 μM) against 10 μM rosiglitazone in the promotion of the PPARγ–LBD–CBP (ligand-binding domain and cAMP-response-element binding protein) interaction as investigated by yeast two-hybrid technology based assay. Molecular modeling studies for compounds **1a–d**, **1h**, **9c–d**, and **13a** were also presented.

Keywords: Difficult sequence; Dipeptide; PPAR; SAR; Tryptophan

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the family of ligand-inducible nuclear receptors [1,2] and have been determined as attractive targets for pharmaceutical intervention of metabolic syndrome [3,4]. PPARs function by forming heterodimers with the 9-*cis*-retinoic acid receptor (RXR) and subsequently binding to specific peroxisome proliferator response elements (PPREs/DR1), thus regulating transcription of their target genes involved in a variety of physiological functions [5]. PPARγ is the most extensively studied among the PPAR subtypes [6], and is believed to be a key regulator of adipocyte differentiation and carbohydrate metabolism [7]. PPARγ plays dual roles in the regulation of insulin sensitivity, one in adipocyte differentiation that contributes to insulin sensitization and the other in adipocyte hypertrophy that leads to insulin resistance, which indicates that both PPARγ agonists and antagonists (also termed ''selective PPARγ modulators, SPPARγM'') are effective in ameliorating insulin sensitivity [8]. Clinical benefits have been achieved through the use of PPARγ agonists – thiazolidinediones (TZDs), such as pioglitazone, rosiglitazone and troglitazone [9]. However, TZDs enhance adipocyte differentiation to improve insulin sensitization, inducing weight gain and edema (fluid retention) [10–12]. PPARγ antagonists are able to reduce triglyceride (TG) content in white adipose tissue, skeletal muscle, and liver [13]. Furthermore, they can also enhance leptin's effects, and increase fatty acid combustion and energy dissipation, thereby ameliorating HF diet-induced obesity and insulin resistance [14–16]. For example, halofenate

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doi:10.1016/j.ejmech.2008.01.032

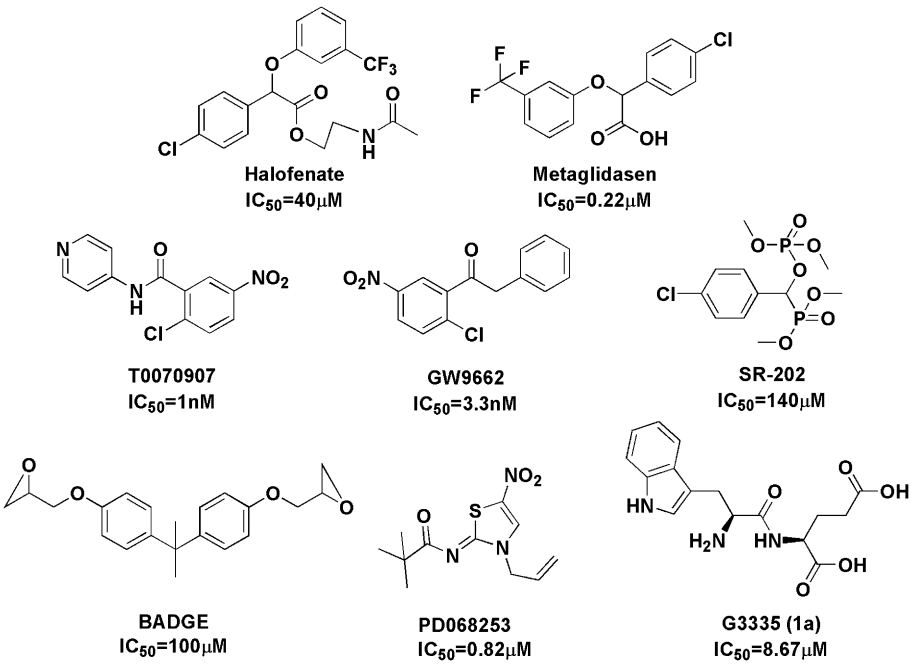


Fig. 1. Structures of lead compound **1a** and the eight known PPARγ antagonists.

($IC_{50} = 40$ μM against 10 μM rosiglitazone, Fig. 1) acts as a PPARγ antagonist in vitro, showing different interactions with cofactors and leading to differential effects on gene expression in a distinct mechanism of action from that of an agonist (rosiglitazone). In the diabetic *ob*/*ob* model, halofenate acutely lowered glucose in a dose-dependent manner [17]. Another PPARγ antagonist metaglidasen [18,19] (also termed MBX-102, Metabolex Inc.) is now in phase III clinical trials, whose profile of lowering glucose, lipids, and uric acid while avoiding weight gain and edema, clearly differentiates metaglidasen from TZDs. PPARγ antagonist T0070907 ($IC_{50} = 1$ nM against 5 nM rosiglitazone) [20] (Fig. 1) blocks fat accumulation in isolated human adipocytes, and is now being developed for the potential treatment of obesity in preclinical trials. Some other PPARγ antagonists have been discovered, such as GW9662 ($IC_{50} = 3.3$ nM against 100 nM rosiglitazone) [21], SR-202 ($IC_{50} = 220$ μM against 5 μM troglitazone) [22], BADGE ($IC_{50} = 100$ μM against 100 nM rosiglitazone) [23], and PD068235 ($IC_{50} = 0.82$ μM against 8 μM rosiglitazone) [24] (Fig. 1). In fact, the discovery of PPARγ antagonists (also termed "selective PPARγ modulators, SPPARγM") is now of special importance in the treatment of diabetes and obesity [25–27].

In our published paper [28], we successfully developed a computational virtual screening approach to search for new PPARγ antagonists from large database, MDL Available Chemicals Directory ACD (www.mdl.com) and SPECS (http://www.specs.net). Twenty-two compounds were found through the virtual screening approach to show binding affinities to PPARγ at submicromolar or micromolar level according to Biacore 3000 results based on the surface plasmon resonance (SPR) technique. According to binding potency, structural similarity, and drug-like analysis, compound **1a** (G3335, Fig. 1) was selected from these twenty-two compounds by virtual screening as the starting point for further structural optimization [28]. Herein, we report the synthesis, biological evaluation, and molecular modeling of a series of novel dipeptides and their analogues. Thirty-five analogues (**1b–l**, **9a–d**, **13a–t**) were synthesized and evaluated against the agonistic effects exerted by rosiglitazone. Six compounds (**1b–c**, **9a–d**) showed strong PPARγ antagonistic activities (IC_{50} values of 5.2–25.8 μM) in antagonizing 10 μM rosiglitazone in the promotion of the PPARγ–LBD–CBP interaction by yeast two-hybrid assay. Moreover, all the synthetic compounds were evaluated by FlexX docking and investigated by molecular modeling docking with PPARγ–LBD, providing possible binding modes at atomic level. Based on these findings, the structure–activity relationships (SARs) of **1a** and its analogues were explored for further development of these novel dipeptide class PPARγ antagonists.

2. Chemistry

2.1. Design of analogues of compound *1a*

A series of 35 novel analogues (**1b–l**, **9a–d**, **13a–t**) were designed and synthesized on the basis of the structural feature of the screening hit **1a**, which exhibited potent PPARγ antagonist activities recently published by Ye et al. [28]. Ye presented that residues Cys285, Arg288, Ser289, and His449 in the active site of PPARγ play important roles in PPARγ–LBD–**1a** binding models. Shortening/lengthening the side chain of the C-terminus of dipeptide **1a**, and/or replacing the indole group of the N-terminus with phenyl or naphthyl ring, we obtained compounds **1b–f** (Table 1) to detect and optimize the ligand-binding modes with residues Arg288,

Table 1
Chemical structures together with the FlexX scores of compounds (**1a–l**, **9a–9d**, **13a–13t**) and their activities

Compound	Structure	FlexX score (kcal/mol)	% Inhibition at 10 mM[a]
1a	H-L-Trp-L-Glu-OH	−23.3	43.2
1b	H-L-Trp-L-Asp-OH	−21.0	57.8
1c	H-L-Trp-L-Aad-OH[b]	−19.9	70.0
1d	H-L-Phe-L-Asp-OH	−20.9	1.12
1e	H-L-Phe-L-Glu-OH	−22.7	4.3
1f	H-L-2-Nal-L-Glu-OH[c]	−23.7	2.1
1g	H-D-Trp-L-Asp-OH	−22.6	0.68
1h	H-D-Trp-D-Asp-OH	−22.7	0.42
1i	H-L-Trp-D-Asp-OH	−21.6	15.5
1j	H-L-Trp-D-Glu-OH	−24.8	0.40
1k	H-D-Trp-L-Glu-OH	−23.5	6.60
1l	H-D-Trp-D-Glu-OH	−23.5	26.7
9a	H-L-Trp-L-Gln-OH	−26.7	38.6
9b	H-L-Trp-L-Asn-OH	−26.2	36.3
9c	H-L-Trp-L-Arg-OH	−18.7	45.0
9d	H-L-Trp-L-Leu-OH	−21.6	58.2
13a	H-L-Trp-L-Asp(OBzl)-OBzl	−18.4	5.33
13b	H-L-Trp-L-Glu(OMe)-OMe	−16.7	4.05
13c	H-L-Trp-L-Asp(OMe)-OMe	−14.9	NI[d]
13d	H-L-Trp-L-Aad(OMe)-OMe	−15.1	8.74
13e	H-L-Trp-L-Leu-OBzl	−17.7	NI[d]
13f	H-L-Trp-L-Leu-OMe	−20.0	5.29
13g	H-L-2-Nal-L-Glu(OBzl)-OBzl	−12.3	27.7
13h	H-L-Trp-D-Glu(OMe)-OMe	−17.6	NI[d]
13i	H-L-Phe-L-Asp(OBzl)-OBzl	−16.3	NI[d]
13j	H-L-Phe-L-Glu(OMe)-OMe	−10.8	3.99
13k	H-L-2-Nal-L-Glu(OMe)-OMe	−13.2	14.7
13l	H-L-Trp-D-Glu(OBzl)-OBzl	−15.3	NI[d]
13m	H-L-Phe-L-Glu(OBzl)-OBzl	−15.3	NI[d]
13n	H-L-2-Nal-L-Leu-OMe	−14.2	4.34
13o	H-D-Phe-L-Aad(OMe)-OMe	−12.9	NI[d]
13p	H-D-Phe-L-Leu-OMe	−14.4	NI[d]
13q	H-D-Phe-L-Asp(OMe)-OMe	−15.0	NI[d]
13r	H-D-Phe-L-Glu(OMe)-OMe	−11.2	NI[d]
13s	H-L-Tyr(*O*-methyl)-L-Glu(OMe)-OMe	−13.2	5.26
13t	H-L-Phe-L-Ala-OMe	−14.0	NI[d]

[a] Data are means of three independent experiments.
[b] Aad, L-2-aminoadipic acid.
[c] Nal, L-2-naphthylalanine.
[d] NI, no inhibition.

Ser289, and His449 in PPARγ. According to the results of molecular modeling, the two nitrogen atoms on the asymmetric centers of **1a** forming hydrogen bonds with Cys285 [28], the isomers **1g–l** (Table 1) of **1a** and **1b** were designed to explore the configuration required by the residue Cys285 in PPARγ. On replacing the carboxylic acid in the side chain of the C-terminus with amide, guanidyl or isobutyl group, compounds **9a–d** (Table 1) were obtained to explore the hydrogen bond formation properties. To improve the lipophilicity and stability, 20 corresponding esterified derivatives (**13a–t**) were designed and synthesized.

2.2. Synthetic methods

Scheme 1 depicts the sequence of reactions that led to the preparation of compounds **1a–l** via traditional strategy, using corresponding L- or D-amino acids as the starting materials. In general, treatment of commercially available L- or D-amino acids (1 equiv) with benzyl alcohol (6.5 equiv) catalyzed by toluene-4-sulfonic acid monohydrate (1.25 equiv) afforded dibenzylated compound **3**. Then compound **3** reacted with **4** to form compounds **5a–l**, and *N*-hydroxylbenzotriazole (HOBt) was employed as an anti-racemization reagent. The deprotection of **5a–l** was easily conducted in one-pot to afford dipeptides **1a–l** through catalytic hydrogenation on Pd/C with bubbling H_2 in quantitative yield.

Compounds **9a–d** were synthesized through the route outlined in Scheme 2. Compound **6** was prepared according to Wilchek's method [29] using DCC to couple *N*-carbobenzoxyl-L-tryptophan and *p*-nitrophenol (HONP). Compound **6** of 1 equiv in THF treated with 1.3 equiv of corresponding amino acids afforded compounds **8a–d**, which could be purified by silica gel chromatography, and then followed by hydrogenation to yield **9a–d** quantitatively.

Scheme 3 depicts the sequence of reactions that led to the preparation of compounds **13a–t** using Boc protected amino acids (**10a–e**) and amino acid esters (**11a–j**) as starting materials. Compounds **12a–t** were synthesized by coupling **10a–e** with **11a–j** using EDCI/HOBt in CH_2Cl_2/DMF at room temperature, and then were deprotected by TFA/triisopropylsilane (TIPS) to afford compounds **13a–t**.

2.3. Molecular docking

To investigate PPARγ antagonists–PPARγ–LBD interaction, homology modeling and molecular docking were employed. The static conformation of PPARγ–LBD binding to **1a** was constructed by homology modeling with the homology module contained in the Insight II software package (based on templates 1KKQ [30] and 2PRG [31]) [28,32]. All compounds (**1a–l**, **9a–d**, **13a–t**) were calculated using FlexX docking to evaluate their binding potency.

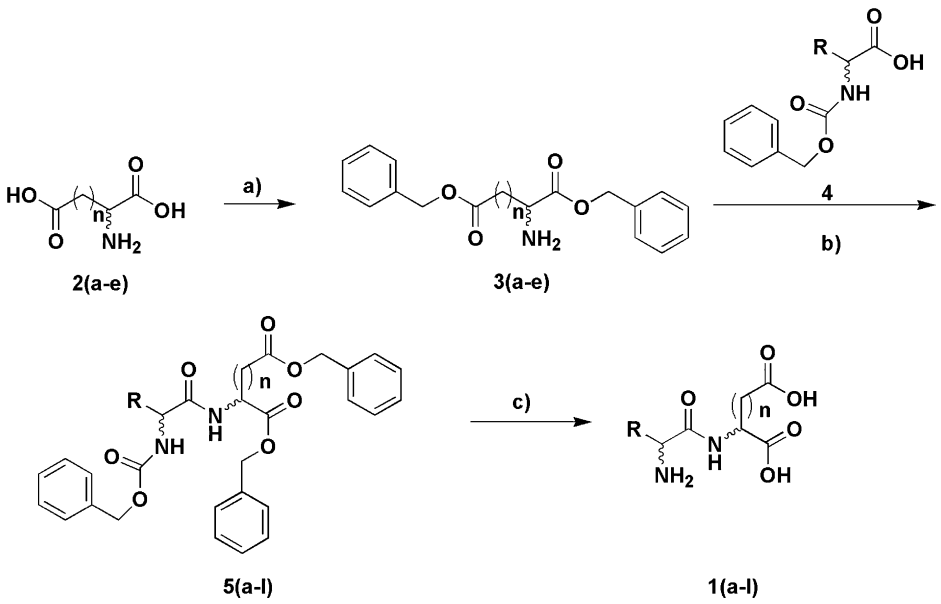
3. Results and discussions

3.1. Analogues' design and synthesis

Thirty-five compounds (**1b–l**, **9a–d**, **13a–t**) were designed and synthesized, and their chemical structures are shown in Table 1. These compounds were synthesized through the routes outlined in Schemes 1–3 via classical strategies, and the details of synthetic procedures and structural characterization are described in Section 5.

3.2. Biological activities

We have exploited an efficient approach in the discovery of PPARγ agonists and antagonists by the yeast two-hybrid system, based on the fact that PPARγ interacts with the coactivator CBP in ligand-dependent fashion [34]. We successfully employed the MEL1 reporter gene to evaluate the protein–protein interactions by conducting a convenient α-galactosidase assay in the AH109 yeast strain with genes for PPARγ–LBD and

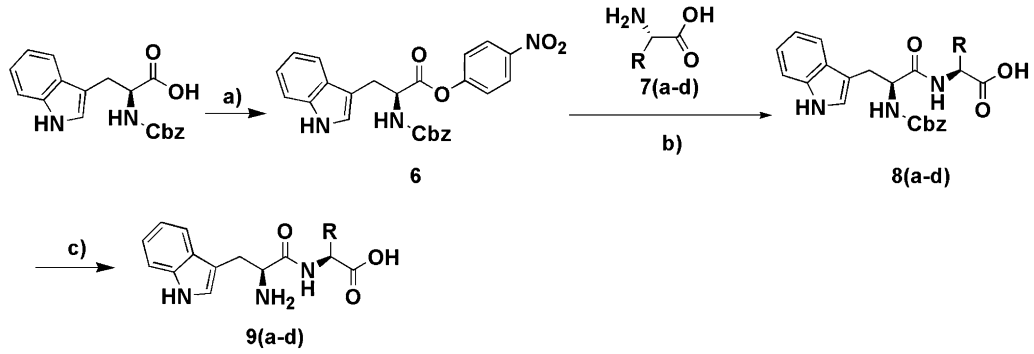


Scheme 1. Synthetic routes of compound **1a–l**. Reagents and conditions: (a) benzyl alcohol, toluene-4-sulfonic acid monohydrate (1.25 equiv), benzene, reflux; (b) EDCI (1.05 equiv), HOBt (1 equiv), TEA (10 equiv), CH_2Cl_2/DMF (10/1, v/v), rt, overnight; (c) H_2, Pd/C, MeOH/H_2O/TIPS (80/20/1, v/v), rt, 8 h.

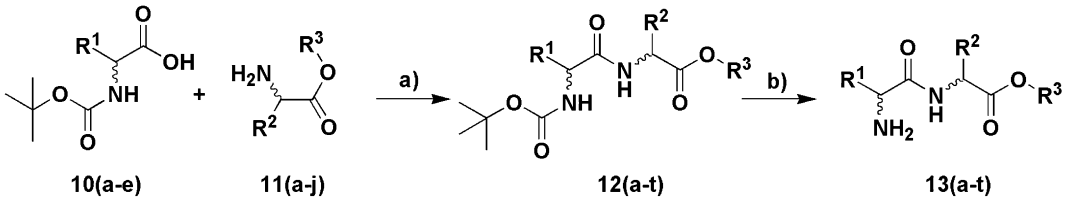
CBP introduced at the N-terminus. The activated MEL1 reporter gene (initiated by ligand and PPARγ activation) expresses α-galactosidase which would hydrolyze PNP-α-Gal into *p*-nitrophenol and D-galactose for detection. For the primary assay, the percent antagonistic activities of compounds **1a–l**, **9a–d**, **13a–t** at the concentration of 10 μM blocking rosiglitazone (10 μM) in the stimulation of the PPARγ–LBD–CBP interaction were measured. The results are summarized in Table 1. The details for bioassay procedures are described in Section 5. Six new compounds (**1b–c**, **9a–d**) can remarkably block rosiglitazone in the stimulation of the PPARγ–LBD–CBP interaction (percent inhibition at 10 μM > 35%), indicating that these six compounds are potent PPARγ antagonists. To determine the exact potency of the compounds that exhibited significant antagonistic activities, six compounds (**1b–c**, **9a–d**) were further investigated in concentration–response studies, and the results are summarized in Fig. 2. Compounds **1b–c** and **9a–d** showed antagonistic activities with IC_{50} values of 5.2, 15.1, 18.7, 18.4, 15.4, and 25.8 μM against rosiglitazone (10 μM), respectively.

3.3. Structure–activity relationship (SAR)

The 3D structure of PPARγ–LBD itself indicates that the antagonistic binding site of PPARγ–LBD consists of three subsites (A–C, Fig. 3a–h), which form a T-shaped groove: site A is a small light hydrophobic binding pocket formed by Ser289, Val290, His323, Ile326, Leu330, Val339, His449, and Tyr473. Right to site A, there is a shallow larger hydrophilic pocket, which is called site B, formed by Phe282, Gln286, Arg288, and Met364. On the opposite side to site B, a deep hydrophobic cavity called site C connects sites A and B, which is composed of Cys285, Tyr327, Ile445, Val446, His449, Leu453, and Tyr473. The binding models indicate that active compounds (**1a–c**, **9c–d**, Figs. 3a–c, f, g and 4a–c, f, g) interact with PPARγ–LBD in a similar way obviously differing from that of inactive compounds (**1d**, **1h** and **13a**, Figs. 3d, e, h and 4d, e, h). The indole group, side chain of C-terminus, and 1-free amino acid group (in the C-terminus) of this dipeptide class antagonists occupy subpockets B, C and A, respectively, contributing a lot of features for



Scheme 2. Synthetic routes of compounds **9a–d**. Reagents and conditions: (a) DCC (1 equiv), HONP (1.02 equiv), THF; (b) TEA (2 equiv), H_2O/THF (1/1, v/v), rt, overnight; (c) H_2, Pd/C, MeOH/H_2O/TIPS (80/20/1, v/v), rt, 8 h.



Scheme 3. Synthetic routes of compounds **13a–t**. Reagents and conditions: (a) EDCI (1 equiv), HOBt (1 equiv), TEA (10 equiv), CH_2Cl_2/DMF (10/1, v/v), rt, overnight; (b) TFA/CH_2Cl_2/TIPS (1/40/0.4, v/v), 0 °C to rt, 12 h.

the binding interaction on the basis of molecular docking characteristics and biological activities.

As shown in Table 1, the initial compound **1a** exhibited 43.2% inhibition rate at the concentration of 10 μM against rosiglitazone (10 μM) in the yeast two-hybrid assay. Shortening/lengthening the side chain of C-terminus of **1a** (compounds **1b** and **1c**) both improved the antagonistic activities to 57.8 and 70.0%, respectively (Table 1). In Figs. 3 and 4, compound **1b** (Figs. 3b and 4b) shows more hydrogen bonds than those of **1a** (Figs. 3a and 4a), and compound **1c** (Figs. 3c and 4c) deserves rich hydrophobic contacts with Phe282, Arg288, Leu453, and Leu465. Thus, the antagonistic activities of compounds have the order of **1c** > **1b** > **1a** (Table 1).

The indole group of compound **1a** forms favorite hydrophobic interaction in subpocket B. Replacing the indole group with phenyl or naphthyl group resulted in a great loss of antagonistic activities (**1d–f**, Table 1), which indicates that the indole group of compound **1a** is preferred at the N-terminus for the antagonistic activity. Actually, the docked conformations of **1d** indicate that the hydrophobic subpocket B can be perfectly occupied by the indole group, but not by the phenyl group (Figs. 3d and 4d).

The diastereomeric configuration of the dipeptide scaffold is essential for its activity. Compounds **1g–l**, the isomers of **1a** or **1b**, presented less to little antagonistic effects than the (*S*,*S*) isomer of **1a** or **1b** (shown in Table 1). These data demonstrate that the stereo requirement for the interaction is strict. The binding mode of (*R*,*R*)-**1h**, an isomer of (*S*,*S*)-**1b**, suggests that the binding features are highly similar to **1b**, except that the C-terminal side chain of dipeptide **1h** stretches into the subpocket A rather than the subpocket C (Figs. 3e and 4e). Such stereo difference results in the loss of inhibitory activity. Moreover, the C-terminal side chain of these dipeptides could play another important role in hydrophobic patch to subpocket C (**9c–d**, Figs. 3c, d and 4c, d). In the side chain of C-terminus, polar groups (such as carboxylic acid, amide, guanidyl groups, **1a–b**, **9a–c**) or non-polar groups (such as isopropyl group, **9d**) retained antagonistic activities. The isopropyl group with higher inhibition rate (58.2%) (Table 1) suggests that smaller hydrophobic group easily enters the deep hydrophobic subpocket C of PPARγ–LBD to exert stronger antagonistic activity (**9d**, Fig. 3d).

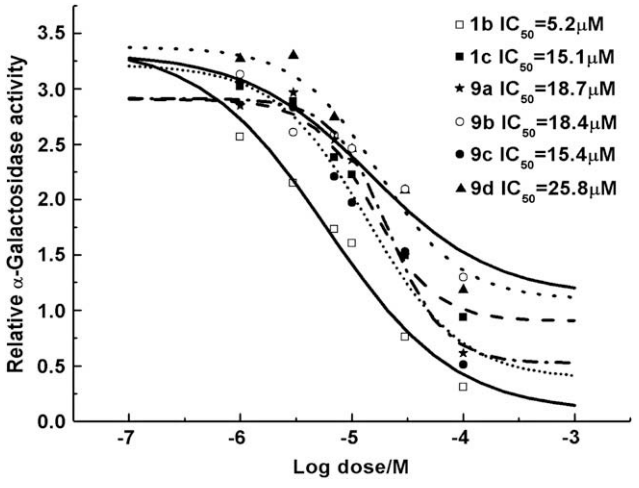


Fig. 2. Concentration dependence of relative α-galactosidase activity by **1b–c** and **9a–d** as determined by yeast two-hybrid assay. The concentration of rosiglitazone was kept constant at 10 μM, while the concentration of compounds ranged from 1 to 100 μM.

Compounds **13a–t** own significantly higher binding energies as shown in Table 1. This is mainly because the blocking of the benzyl or methylester group (**13a–t**) will impair the capability of carboxyl group to form hydrogen bond. Compounds **13a–t**, in which 1-carboxyl acid protected by benzyl or methylesters, showed decreased antagonistic activities in the assay, and many of them were proved to completely lose the antagonistic activity (shown in Table 1). 1-Free carboxylic acid of the C-terminus of dipeptide **1a** forms hydrogen bonds with residues Ser289, His323, and His449 in subpocket A. In the binding modeling using compound **13a** (Fig. 3h) as an example, the ester groups on the C-terminal of these dipeptides are too large to be inserted into the subpockets A and C. Moreover, the ester group on the C-terminal cannot form hydrogen bonds with Ser289 and His323 any more. As a result, the binding conformation of **13a** is far away from the subpocket A and subpocket C, and therefore losing antagonistic activity. These demonstrate that 1-free carboxylic acid of the C-terminus is preferred in the further antagonist optimization.

4. Conclusions

In summary, a series of novel PPARγ antagonists (also termed "selective PPARγ modulators, SPPARγM") were designed and synthesized based on the initial hit **1a**. All compounds were evaluated by yeast two-hybrid assay for their PPARγ antagonistic activities. Selected compounds for concentration–response studies showed prominent antagonistic activities with IC_{50} values ranging from 5.2 to 25.8 μM. Basing on successful homology modeling of PPARγ, binding

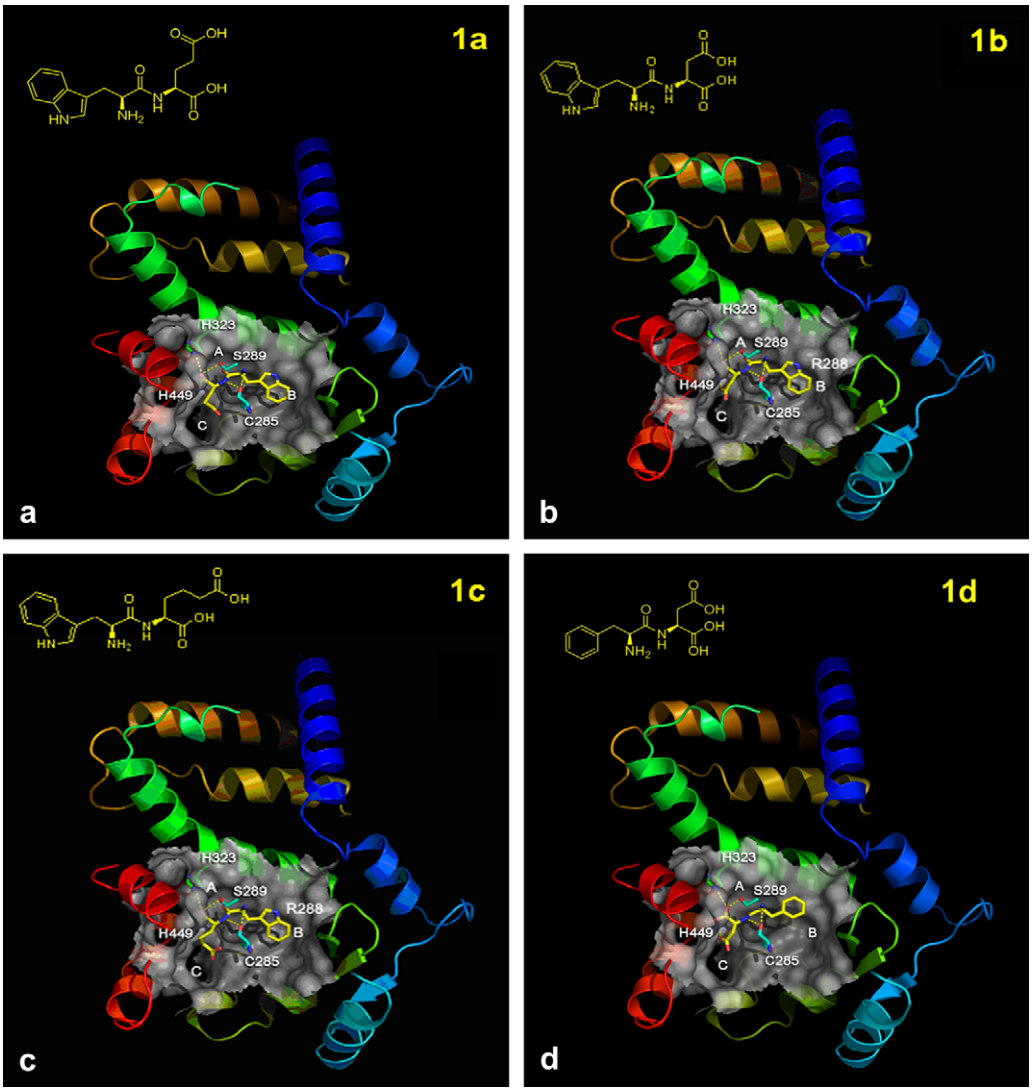


Fig. 3. Three-dimensional binding modes of **1a** (a), **1b** (b), **1c** (c), **1d** (d), **1h** (e), **9c** (f), **9d** (g) and **13a** (h) to PPARγ–LBD derived from the docking simulations. Images were generated using Pymol program (http://www.pymol.org).

affinity was evaluated by FlexX, and SARs were explored. Further studies are in progress.

5. Experimental section

5.1. Yeast two-hybrid based assay

The yeast two-hybrid system used for PPARγ antagonist screening was constructed by the method published by Chen et al. [34]. The MEL1 reporter gene (the GAL gene family member) was used in the yeast two-hybrid system for detection of protein–protein interactions through conducting α-galactosidase assays [28,35]. By the PCR technique, mCBP (aa 1–464) and hPPARγ–LBD (aa 204–477) were inserted into the vectors pGADT7 and pGBKT7 (Clontech), respectively, to provide pGADT7–CBP and pGBKT7–PPARγ plasmids, which were then co-transfected into yeast strain AH109 by the lithium acetate method [35]. The corresponding yeast cells were cultured in SD minimal medium (3 mL) without leucine and tryptophan (T^-L^-) (yeast nitrogen base without amino acids 6.7 g L^{-1}, D-(+)-glucose 20 g L^{-1}, yeast synthetic drop-out medium supplement without leucine and tryptophan 1.54 g L^{-1}) at 30 °C with shaking (250 rpm). When the OD_{600} of the cells had reached about 1.0, the cultures were diluted 20 times with fresh T^-L^- medium, and different concentrations of the 35 compounds synthesized in this work (10 μL) with rosiglitazone (10 mM, 1 μL) or DMSO (11 μL, as a blank) were then added to diluted cultures (989 μL). After further incubation at 30 °C for 16 h, each sample (200 μL) was transferred to a 96-well plate (Corning Costar 96-well flat-bottomed plate), and the OD_{600} value was measured with a Benchmark Plus™ microplate spectrophotometer (Bio-Rad). The cells were then centrifuged and the supernatant (16 μL) was transferred to another 96-well plate with assay buffer (48 μL; 16 μL of 100 mM PNP-α-Gal + 32 μL of 0.5 M sodium acetate, pH 4.5 per well). After a further 60 min incubation at 30 °C, the reaction was terminated by the addition of stop solution (136 μL, 1 M Na_2CO_3) in each

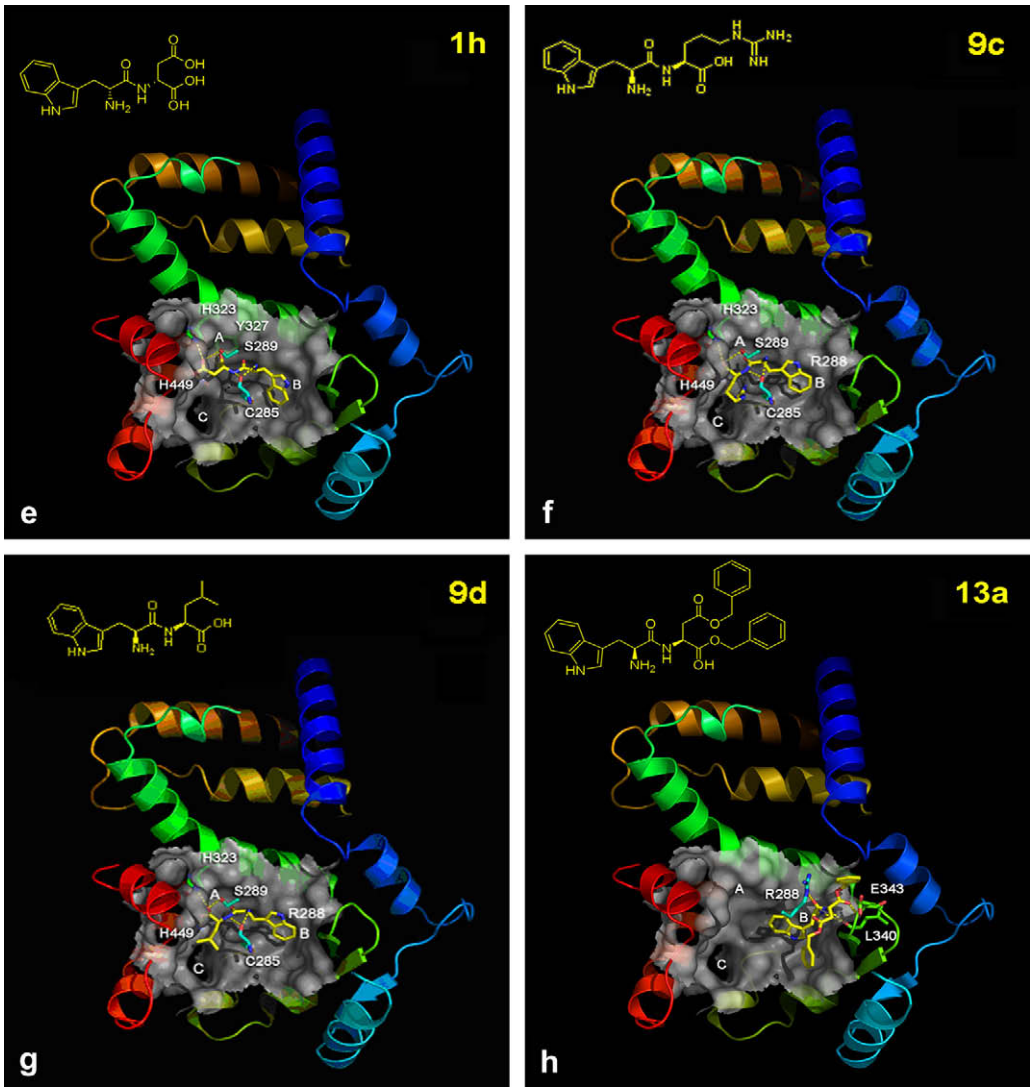


Fig. 3. (*Continued*).

well, and the OD_{410} was measured. One unit of α-galactosidase is defined as the amount of enzyme that hydrolyzes 1 mmol PNP-α-Gal to *p*-nitrophenol and D-galactose in 1 min at 30 °C in acetate buffer (pH 4.5) [36]. The α-galactosidase activity in milliunits per well was calculated according to the following formula:

$$\alpha\text{-Galactosidase activity (milliunits)} = OD_{410} \times V_f \times 1000/[(\varepsilon \times b) \times t \times V_i \times OD_{600}]$$

In the formula, t is the elapsed time of incubation (min), V_f is the final volume of assay (200 μL), V_i is the volume of culture medium supernatant added (16 μL), OD_{600} is the optical density of the cells at the start of the assay, and $\varepsilon \times b$ is *p*-nitrophenol molar absorptivity at 410 nm × the light path (cm) = 10.5 (mL μmol^{-1}; Yeast Protocols Handbook PT3024-1, Clontech).

The inhibition assays of compounds (**1b–c**, **9a–d**) were performed in the same manner as mentioned above. The overnight cultures of yeast cells containing pGADT7–CBP and pGBKT7–PPARγ were diluted with fresh media to an initial OD_{600} of 0.05 and treated with rosiglitazone (10 μM) and increasing concentrations of the six compounds or DMSO (as vehicle control) for 16 h at 30 °C. Relative α-galactosidase activity was measured on the cells treated with 1% DMSO. To calculate the half-maximal antagonistic concentration (IC_{50}), the relative α-galactosidase activity is plotted against the common logarithm of the compound concentration, and the data were fitted using the sigmoidal fitting model by the Origin7.0 software.

5.2. Molecular modeling

The 3D structures of compounds were constructed with the standard geometric parameters of the SYBYL 6.8 molecular modeling software package (http://www.tripos.com). The geometries of these antagonists were subsequently optimized by the Power method encoded in SYBYL 6.8. The Tripos force field and Gasteiger–Hückel charges were employed during the antagonist minimization [37]. As described in our previous report [28], 3D model of PPARγ–LBD was generated by homology modeling, based on 1KKQ [30] and 2PRG [31]. The structure-conserved regions of the two template

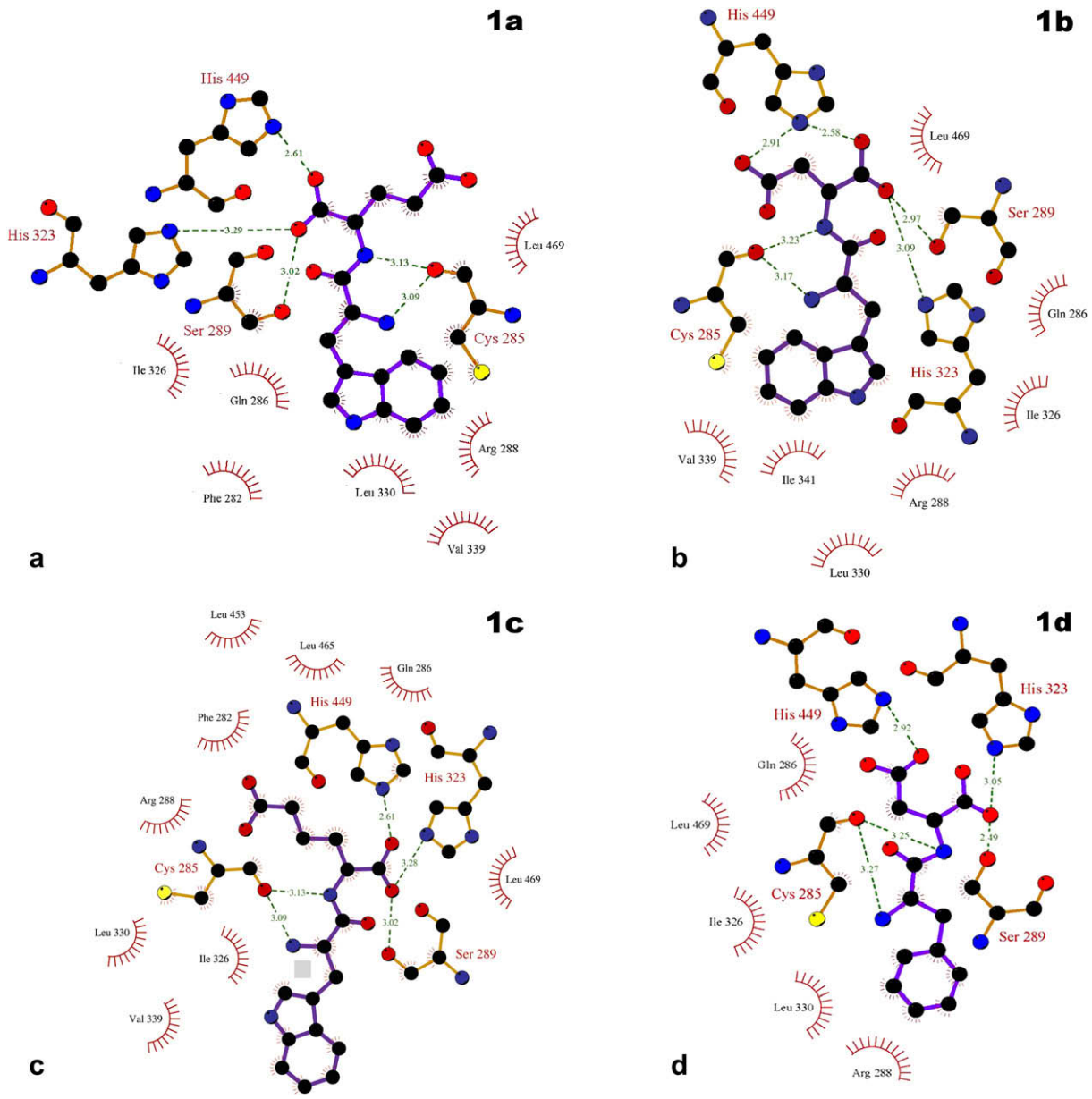


Fig. 4. Two-dimensional representation for the interacting mode of compounds **1a** (a), **1b** (b), **1c** (c), **1d** (d), **1h** (e), **9c** (f), **9d** (g) and **13a** (h) with PPARγ–LBD as plotted with LIGPLOT program [33]. Dashed lines represent H-bonds and spiked residues represent hydrophobic contacts with the ligands.

proteins were determined by structure alignment of the homology module in Insight II [32]. The final model protein was validated both by Procheck and by Profiles-3D [38,39]. All compounds (**1a–l**, **9a–d**, **13a–t**) were docked into the constructed PPARγ–LBD to obtain the corresponding binding energies by FlexX (incorporated in SYBYL 6.8).

All the molecular modeling and docking simulations were performed on a Silicon Graphics Origin3200 workstation (with four CPUs).

5.3. Chemistry

The reagents were purchased from Alfa Aesar, Acros, Sigma–Aldrich, Shanghai Chemical Reagent Company, and GL Biochem and were used without further purification. Analytical thin-layer chromatography (TLC) was HSGF 254 silica gel (0.15–0.2 mm thickness, Yantai Huiyou Company, China). CH_2Cl_2 was distilled from CaH_2 and DMF was purchased in HPLC grade. Solvents were evaporated under reduced pressure and below 50 °C if no description was noted. Melting points were measured in capillary tube with an SGW X-4 melting point apparatus without correction. Rotary nuclear magnetic resonance (NMR) spectra were obtained on a Brucker AMX-300 NMR (TMS as IS). Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane (TMS). Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (LRMS and HRMS) were given with electric and electrospray ionization (EI, ESI) produced, respectively, by Finnigan

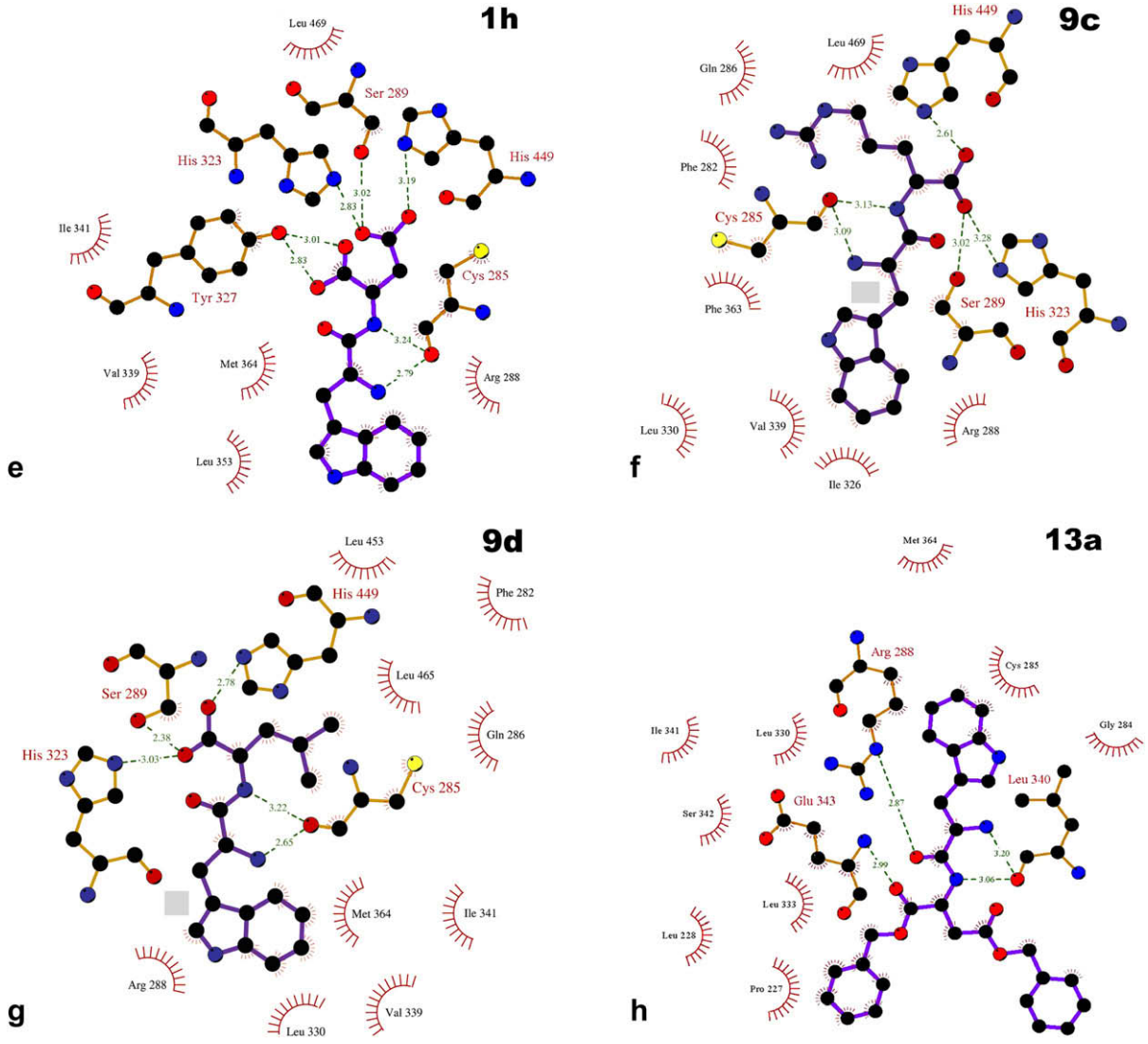


Fig. 4. (*Continued*).

MAT-95, LCQ-DECA spectrometer, and IonSpec 4.7 T. Degrees of optical activity were measured with PE-341 polarimeter.

5.4. General procedures for preparation of *3a–e* using *3a* as an example

5.4.1. L-Dibenzylester glutamate p-toluenesulfonate (*3a*)

A mixture of L-glutamic acid (4 g, 27 mmol), *p*-toluenesulfonic acid (6.445 g, 34 mmol), benzyl alcohol (18 mL) and benzene (27 mL) was heated to reflux for 4 h with vigorous stirring, then benzene was removed under reduced pressure and fresh benzene was added to reflux for another 1 h. This procedure was repeated three times. Then ethyl ether (50 mL) was added to form a white precipitate and filtered. The filter cake was washed with ethyl ether and dried under high vacuum to give 12.5 g (92%) of **3a** as a white solid. ^{1}H NMR (CD_3OD): $\delta = 7.69$ (d, 2H), 7.36 (m, 10H), 7.22 (d, 2H), 5.24 (s, 2H), 5.10 (s, 2H), 4.15 (d, 1H), 2.52 (m, 2H), 2.35 (s, 3H), 2.17 (m, 2H).

5.4.2. L-Dibenzylester aspartate p-toluenesulfonate (*3b*)

Compound **3b** was prepared from L-aspartic acid using a procedure similar to that described for the preparation of **3a** as a white solid, yield 90%. ^{1}H NMR (DMSO-d_6): $\delta = 7.49$ (d, 2H), 7.37 (m, 10H), 7.12 (d, 2H), 5.19 (s, 2H), 5.11 (s, 2H), 4.49 (t, 1H), 3.02 (m, 2H), 2.29 (s, 3H).

5.4.3. L-2-Aminoadipic acid dibenzylester p-toluenesulfonate (*3c*)

Compound **3c** was prepared from L-2-aminoadipic acid using a procedure similar to that described for the preparation of **3a** as a white solid, yield 87%. ^{1}H NMR (DMSO-d_6): $\delta = 7.55$ (d, 2H), 7.38 (m, 10H), 7.15 (d, 2H), 5.18 (s, 2H), 4.97 (s, 2H), 4.49 (t, 1H), 2.42 (m, 2H), 2.29 (s, 3H), 1.92 (m, 2H), 1.71 (m, 2H).

5.4.4. D-Dibenzylester glutamate p-toluenesulfonate (*3d*)

Compound **3d** was prepared from D-glutamic acid using a procedure similar to that described for the preparation of **3a** as a white solid, yield 83%. ^{1}H NMR (CD_3OD): $\delta = 7.70$

(d, 2H), 7.36 (m, 10H), 7.22 (d, 2H), 5.24 (s, 2H), 5.09 (s, 2H), 4.14 (d, 1H), 2.52 (m, 2H), 2.35 (s, 3H), 2.17 (m, 2H).

5.4.5. D-Dibenzylester aspartate p-toluenesulfonate (3e)

Compound **3e** was prepared from D-aspartic acid using a procedure similar to that described for the preparation of **3a** as a white solid, yield 94%. ^{1}H NMR (DMSO-d_6): $\delta = 7.43$ (d, 2H), 7.37 (m, 10H), 7.15 (d, 2H), 5.19 (s, 2H), 5.11 (s, 2H), 4.46 (t, 1H), 3.01 (m, 2H), 2.28 (s, 3H).

5.5. General procedures for preparation of 5a–l

5.5.1. N-Carbobenzoxyl-L-tryptophan-L-glutamate dibenzylester (5a)

To a mixture of L-dibenzylester glutamate *p*-toluenesulfonate (**3a**) (0.5 g, 1 mmol), *N*-carbobenzoxyl-L-tryptophan (0.34 g, 1 mmol), 1-hydroxybenzotriazole (HOBt, 0.14 g, 1 mmol) and triethylamine (1.4 mL, 10 mmol) in 5% DMF/CH_2Cl_2 (20 mL), 1-ethyl-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI, 0.2 g, 1.05 mmol) was added in portions for 5 min. The solution was stirred overnight at room temperature (rt) followed by TLC detection. The solution was washed, dried, filtered, and condensed to form a yellow oil. The oil can be purified by flash chromatography with petroleum ether/ethyl acetate (4/1, v/v) to afford a white powder (0.59 g, 91%). Mp 98–99 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.82$ (s, 1H), 7.62 (d, 1H), 7.21–7.40 (m, 16H), 6.95 (s, 1H), 6.38 (d, 1H), 5.43 (d, 1H), 5.15 (m, 2H), 5.06 (s, 2H), 5.03 (s, 2H), 4.52 (m, 2H), 3.39 (dd, 1H), 3.10 (q, 1H), 2.14 (br, 3H), 1.83 (m, 1H).

5.5.2. N-Carbobenzoxyl-L-tryptophan-L-aspartate dibenzylester (5b)

Compound **5b** was prepared from *N*-carbobenzoxyl-L-tryptophan and L-dibenzylester aspartate *p*-toluenesulfonate (**3b**) using a procedure similar to that described for the preparation of **5a** as a white solid, yield 87%. Mp 151–153 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.84$ (s, 1H), 7.62 (d, 1H), 6.95–7.41 (br, 16H), 6.85 (d, 1H), 5.12 (m, 1H), 5.08 (s, 2H), 4.98 (d, 2H), 4.80 (m, 1H), 3.25 (m, 1H), 3.20 (m, 1H), 3.01 (dd, 1H), 2.8 (dd, 1H).

5.5.3. N-Carbobenzoxyl-L-tryptophan-L-2-aminoadipate dibenzylester (5c)

Compound **5c** was prepared from *N*-carbobenzoxyl-L-tryptophan and L-2-aminoadipic acid dibenzylester *p*-toluenesulfonate (**3c**) using a procedure similar to that described for the preparation of **5a** as a white solid, yield 85%. Mp 99–100 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.39$ (s, 1H), 7.65 (d, 1H), 7.10–7.36 (m, 15H), 7.13 (t, 1H), 7.08 (t, 1H), 6.31 (d, 1H), 5.47 (d, 1H), 5.13 (s, 2H), 4.99 (s, 2H), 4.97 (s, 2H), 4.55 (m, 1H), 4.48 (q, 1H), 3.34 (dd, 1H), 3.14 (q, 1H), 2.20 (t, 1H), 1.64 (m, 1H), 1.51 (m, 1H).

5.5.4. N-Carbobenzoxyl-L-phenylalanine-L-aspartate dibenzylester (5d)

Compound **5d** was prepared from *N*-carbobenzoxyl-L-phenylalanine and L-dibenzylester aspartate *p*-toluenesulfonate (**3b**) using a procedure similar to that described for the preparation of **5a** as a white solid, yield 86%. ^{1}H NMR ($CDCl_3$): $\delta = 7.10$–7.41 (m, 20H), 5.12 (s, 2H), 4.99 (s, 2H), 4.97 (s, 2H), 4.22 (t, 1H), 3.98 (m, 1H), 3.23 (m, 1H), 2.91 (q, 1H), 2.28 (m, 2H).

5.5.5. N-Carbobenzoxyl-L-phenylalanine-L-glutamate dibenzylester (5e)

Compound **5e** was prepared from *N*-carbobenzoxyl-L-phenylalanine and L-dibenzylester glutamate *p*-toluenesulfonate (**3a**) using a procedure similar to that described for the preparation of **5a** as a white solid, yield 88%. Mp 100–101 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.08$–7.39 (m, 20H), 6.56 (d, 1H), 5.11 (s, 2H), 5.18 (d, 2H), 5.10 (s, 2H), 4.81 (m, 1H), 4.36 (q, 1H), 3.05 (dd, 2H), 2.22 (m, 3H), 1.98 (m, 1H).

5.5.6. N-Carbobenzoxyl-L-2-naphthylalanine-L-glutamate dibenzylester (5f)

Compound **5f** was prepared from *N*-carbobenzoxyl-L-2-naphthylalanine and L-dibenzylester glutamate *p*-toluenesulfonate (**3a**) using a procedure similar to that described for the preparation of **5a** as a white solid, yield 80%. Mp 110–111 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.73$ (m, 3H), 7.65 (s, 1H), 7.22–7.43 (m, 18H), 5.12 (d, 2H), 5.02 (s, 2H), 4.89 (s, 2H), 4.42 (m, 2H), 3.18 (q, 1H), 2.98 (q, 1H), 2.25 (s, 3H), 1.8 (m, 1H).

5.5.7. N-Carbobenzoxyl-D-tryptophan-L-aspartate dibenzylester (5g)

Compound **5g** was prepared from *N*-carbobenzoxyl-D-tryptophan and L-dibenzylester aspartate *p*-toluenesulfonate (**3b**) using a procedure similar to that described for the preparation of **5a** as a white solid, yield 85%. Mp 116–118 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.63$ (s, 1H), 7.57 (d, 1H), 7.06–7.36 (br, 17H), 6.90 (s, 1H), 6.60 (d, 1H), 5.44 (m, 1H), 5.08 (s, 2H), 5.02 (s, 2H), 4.85 (q, 2H), 4.79 (m, 1H), 4.49 (br, 1H), 3.08–3.27 (m, 2H), 2.83 (dd, 1H), 2.30 (dd, 1H), 1.61 (br, 1H).

5.5.8. N-Carbobenzoxyl-D-tryptophan-D-aspartate dibenzylester (5h)

Compound **5h** was prepared from *N*-carbobenzoxyl-D-tryptophan and D-dibenzylester aspartate *p*-toluenesulfonate (**3e**) using a procedure similar to that described for the preparation of **5a** as a white solid, yield 80%. Mp 155–157 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.79$ (s, 1H), 7.65 (d, 1H), 7.05–7.38 (br, 17H), 6.96 (d, 1H), 6.65 (d, 1H), 5.38 (br, 1H), 5.08 (m, 4H), 4.93 (d, 2H), 4.78 (m, 1H), 4.50 (br, 1H), 3.19 (m, 2H), 2.94 (m, 2H).

5.5.9. N-Carbobenzoxyl-L-tryptophan-D-aspartate dibenzylester (5i)

Compound **5i** was prepared from *N*-carbobenzoxyl-L-tryptophan and D-dibenzylester aspartate *p*-toluenesulfonate (**3e**) using a procedure similar to that described for the preparation of **5a** as a white solid, yield 88%. Mp 115–117 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.64$ (s, 1H), 7.60 (d, 1H), 7.05–7.41 (br, 16H), 6.91 (s, 1H), 6.61 (d, 1H), 5.44 (m, 1H), 5.09 (s, 2H), 5.02 (s,

2H), 4.79 (m, 1H), 4.49 (br, 1H), 3.08–3.16 (m, 2H), 2.83 (dd, 1H), 2.27 (dd, 1H), 1.60 (br, 1H).

5.5.10. N-Carbobenzoxyl-L-tryptophan-D-glutamate dibenzylester (5j)

Compound **5j** was prepared from *N*-carbobenzoxyl-L-tryptophan and D-dibenzylester glutamate *p*-toluenesulfonate (**3d**) using a procedure similar to that described for the preparation of **5a** as a white solid, yield 88%. Mp 54–55 °C; ^{1}H NMR (CD_3OD): $\delta = 7.54$ (d, 1H), 6.90–7.41 (br, 19H), 5.08 (s, 2H), 5.06 (s, 2H), 4.97 (s, 2H), 4.46 (br, 2H), 3.17 (m, 1H), 3.04 (m, 1H), 2.32 (m, 2H), 2.12 (br, 1H), 1.78 (m, 1H).

5.5.11. N-Carbobenzoxyl-D-tryptophan-L-glutamate dibenzylester (5k)

Compound **5k** was prepared from *N*-carbobenzoxyl-D-tryptophan and L-dibenzylester glutamate *p*-toluenesulfonate (**3a**) using a procedure similar to that described for the preparation of **5a** as a white solid, yield 85%. Mp 115–117 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.77$ (s, 1H), 7.65 (d, 1H), 7.05–7.42 (br, 17H), 6.93 (d, 1H), 6.27 (d, 1H), 5.43 (d, 1H), 5.10 (s, 2H), 5.07 (s, 2H), 5.06 (s, 2H), 4.54 (m, 2H), 3.40 (m, 1H), 3.15 (q, 1H), 1.93 (m, 2H).

5.5.12. N-Carbobenzoxyl-D-tryptophan-D-glutamate dibenzylester (5l)

Compound **5l** was prepared from *N*-carbobenzoxyl-D-tryptophan and D-dibenzylester glutamate *p*-toluenesulfonate (**3d**) using a procedure similar to that described for the preparation of **5a** as a white solid, yield 92%. Mp 116–117 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.86$ (s, 1H), 7.62 (d, 1H), 7.08–7.39 (br, 15H), 7.15 (t, 1H), 7.10 (t, 1H), 6.95 (s, 1H), 6.40 (d, 1H), 5.43 (d, 1H), 5.10 (d, 2H), 5.06 (s, 2H), 5.03 (s, 2H), 4.52 (m, 2H), 3.38 (m, 1H), 3.11 (q, 1H), 2.13 (m, 3H), 1.80 (m, 1H).

5.6. General procedures for preparation of 1a–l using compound 1a as an example

5.6.1. L-Tryptophan-L-glutamic acid (1a)

A solution of **5a** (0.59 g, 0.91 mmol) and triisopropylsilane (100 μL) in 80% methanol–water (10 mL) was hydrogenated in the presence of 0.1 g of 10% palladium on charcoal (Pd/C) with stirring for 8 h. The solution was filtered and the filtrate was evaporated to form a light oil. The oil was dissolved in water and washed with ethyl acetate. The water portion was dried by lyophilization to form **1a** (0.3 g, 95%). Mp 116–118 °C; $[\alpha]_D^{20}$ +11.2 (*c* 0.5, water); ^{1}H NMR (DMSO-d_6): $\delta = 7.66$ (d, 1H), 7.33 (d, 1H), 7.21 (s, 1H), 7.06 (t, 1H), 6.95 (t, 1H), 4.21 (m, 1H), 3.93 (m, 1H), 3.21 (dd, 1H), 2.97 (m, 1H), 2.28 (m, 2H), 1.87 (m, 2H); ESI-MS *m/z* 334, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{16}H_{20}N_3O_5$ $[M+H]^+$ 334.1403, found 334.1416.

5.6.2. L-Tryptophan-L-aspartic acid (1b)

Compound **1b** was prepared from *N*-carbobenzoxyl-L-tryptophan-L-aspartate dibenzylester (**5b**) using a procedure similar to that described for the preparation of **1a** as a white solid, yield 98%. Mp 167–168 °C; $[\alpha]_D^{20}$ +20.3 (*c* 0.5, water); ^{1}H NMR (DMSO-d_6): $\delta = 7.46$ (d, 1H), 7.34 (d, 1H), 7.12 (t, 1H), 7.03 (d, 1H), 4.50 (t, 1H), 4.38 (q, 2H), 4.10 (dd, 1H), 3.32 (dd, 1H), 3.01 (q, 1H), 2.74 (m, 2H); ESI-MS *m/z* 320, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{15}H_{18}N_3O_5$ $[M+H]^+$ 320.1232, found 320.1246.

5.6.3. L-Tryptophan-L-2-aminoadipic acid (1c)

Compound **1c** was prepared from *N*-carbobenzoxyl-L-tryptophan-L-2-aminoadipate dibenzylester (**5c**) using a procedure similar to that described for the preparation of **1a** as a white solid, yield 98%. Mp 99–100 °C; $[\alpha]_D^{20}$ +18.9 (*c* 0.5, water); ^{1}H NMR (DMSO-d_6): $\delta = 10.98$ (s, 1H), 8.48 (d, 1H), 7.63 (d, 1H), 7.32 (d, 1H), 7.20 (s, 1H), 7.05 (t, 1H), 6.95 (t, 1H), 4.16 (m, 3H), 3.87 (q, 1H), 3.16 (dd, 1H), 2.96 (q, 1H), 2.23 (m, 2H), 1.83 (m, 2H); ESI-MS *m/z* 348, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{17}H_{22}N_3O_5$ $[M+H]^+$ 348.1559, found 348.1549.

5.6.4. L-Phenylalanine-L-aspartic acid (1d)

Compound **1d** was prepared from *N*-carbobenzoxyl-L-phenylalanine-L-aspartate dibenzylester (**5d**) using a procedure similar to that described for the preparation of **1a** as a white solid, yield 95%. Mp 57–58 °C; $[\alpha]_D^{20}$ +25.1 (*c* 0.5, water); ^{1}H NMR (CD_3OD): $\delta = 8.89$ (d, 1H), 7.28 (m, 5H), 4.50 (t, 1H), 4.08 (q, 1H), 3.14 (dd, 1H), 2.94 (q, 1H), 2.66 (q, 2H); ESI-MS *m/z* 281, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{13}H_{17}N_2O_5$ $[M+H]^+$ 281.1137, found 281.1146.

5.6.5. L-Phenylalanine-L-glutamic acid (1e)

Compound **1e** was prepared from *N*-carbobenzoxyl-L-phenylalanine-L-glutamate dibenzylester (**5e**) using a procedure similar to that described for the preparation of **1a** as a white solid, yield 97%. Mp 226–227 °C; $[\alpha]_D^{20}$ +12.1 (*c* 0.5, water); ^{1}H NMR (DMSO-d_6): $\delta = 7.30$ (m, 3H), 7.17 (q, 2H), 4.25 (q, 1H), 3.68 (t, 1H), 3.06 (m, 2H), 2.31 (t, 2H), 1.98 (m, 1H), 1.80 (m, 1H); ESI-MS *m/z* 295, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{14}H_{19}N_2O_5$ $[M+H]^+$ 295.1294, found 295.1304.

5.6.6. L-2-Naphthylalanine-L-glutamic acid (1f)

Compound **1f** was prepared from *N*-carbobenzoxyl-L-2-naphthylalanine-L-glutamate dibenzylester (**5f**) using a procedure similar to that described for the preparation of **1a** as a white solid, yield 99%. Mp 57–58 °C; $[\alpha]_D^{20}$ +21.1 (*c* 0.5, water); ^{1}H NMR (DMSO-d_6): $\delta = 8.72$ (d, 1H), 7.61 (m, 4H), 7.43 (m, 3H), 4.16 (m, 2H), 3.24 (dd, 1H), 3.20 (q, 1H), 2.19 (t, 2H), 1.8 (m, 2H); ESI-MS *m/z* 345, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{18}H_{21}N_2O_5$ $[M+H]^+$ 345.1450, found 345.1455.

5.6.7. D-Tryptophan-L-aspartic acid (1g)

Compound **1g** was prepared from *N*-carbobenzoxyl-D-tryptophan-L-aspartate dibenzylester (**5g**) using a procedure similar to that described for the preparation of **1a** as a white solid, yield 94%. Mp 161–163 °C, $[\alpha]_D^{20}$ −39.2 (*c* 0.5, water);

^{1}H NMR (DMSO-d_6): $\delta = 10.99$ (d, 1H), 8.56 (d, 1H), 7.67 (d, 1H), 7.33 (d, 1H), 7.19 (s, 1H), 7.08 (t, 1H), 6.98 (t, 1H), 4.23 (m, 1H), 4.06 (m, 1H), 2.91–3.21 (m, 2H), 2.40 (m, 1H), 2.24 (m, 1H); ESI-MS *m/z* 320, $[M + H]^+$; HRMS (ESI) *m/z* calcd $C_{15}H_{18}N_3O_5$ $[M + H]^+$ 320.1246, found 320.1240.

5.6.8. D-Tryptophan-D-aspartic acid (**1h**)

Compound **1h** was prepared from *N*-carbobenzoxyl-D-tryptophan-D-aspartate dibenzylester (**5h**) using a procedure similar to that described for the preparation of **1a** as a white solid, yield 94%. Mp 190–191 °C; $[\alpha]_D^{20}$ −37.8 (*c* 0.5, water); ^{1}H NMR (CD_3OD): $\delta = 7.69$ (d, 1H), 7.36 (d, 1H), 7.25 (s, 1H), 7.04–7.14 (m, 2H), 4.56 (m, 1H), 4.16 (m, 1H), 3.45 (dd, 1H), 3.22 (q, 1H), 2.80 (m, 2H); ESI-MS *m/z* 320, $[M + H]^+$; HRMS (ESI) *m/z* calcd $C_{15}H_{18}N_3O_5$ $[M + H]^+$ 320.1246, found 320.1222.

5.6.9. L-Tryptophan-D-aspartic acid (**1i**)

Compound **1i** was prepared from *N*-carbobenzoxyl-L-tryptophan-D-aspartate dibenzylester (**5i**) using a procedure similar to that described for the preparation of **1a** as a white solid, yield 99%. Mp 163–164 °C; $[\alpha]_D^{20}$ +25.8 (*c* 0.5, water); ^{1}H NMR (CD_3OD): $\delta = 7.64$ (d, 1H), 7.35 (d, 1H), 7.19 (s, 1H), 7.10 (m, 2H), 4.58 (t, 1H), 4.10 (t, 1H), 3.33 (m, 1H), 3.22 (m, 1H), 2.54 (m, 2H); ESI-MS *m/z* 320, $[M + H]^+$; HRMS (ESI) *m/z* calcd $C_{15}H_{18}N_3O_5$ $[M + H]^+$ 320.1246, found 320.1240.

5.6.10. L-Tryptophan-D-glutamic acid (**1j**)

Compound **1j** was prepared from *N*-carbobenzoxyl-L-tryptophan-D-glutamate dibenzylester (**5j**) using a procedure similar to that described for the preparation of **1a** as a white solid, yield 99%. Mp 49–50 °C; $[\alpha]_D^{20}$ −45.2 (*c* 0.5, water); ^{1}H NMR (DMSO-d_6): $\delta = 7.70$ (d, 1H), 7.34 (d, 1H), 7.20 (s, 1H), 7.07 (t, 1H), 7.00 (t, 1H), 4.20 (m, 1H), 3.99 (m, 1H), 3.28 (m, 1H), 3.08 (m, 1H), 2.14 (t, 2H), 1.80 (m, 2H); ESI-MS *m/z* 334, $[M + H]^+$; HRMS (ESI) *m/z* calcd $C_{16}H_{20}N_3O_5$ $[M + H]^+$ 334.1403, found 334.1393.

5.6.11. D-Tryptophan-L-glutamic acid (**1k**)

Compound **1k** was prepared from *N*-carbobenzoxyl-D-tryptophan-L-glutamate dibenzylester (**5k**) using a procedure similar to that described for the preparation of **1a** as a white solid, yield 91%. Mp 169–170 °C; $[\alpha]_D^{20}$ −85.2 (*c* 0.5, water); ^{1}H NMR (DMSO-d_6): $\delta = 10.94$ (d, 1H), 8.30 (d, 1H), 7.63 (d, 1H), 7.32 (d, 1H), 7.17 (s, 1H), 7.02 (t, 1H), 6.98 (t, 1H), 4.08 (m, 2H), 3.13 (dd, 1H), 2.93 (q, 1H), 2.11 (m, 2H), 1.71 (m, 2H); ESI-MS *m/z* 334, $[M + H]^+$; HRMS (ESI) *m/z* calcd $C_{16}H_{20}N_3O_5$ $[M + H]^+$ 334.1403, found 334.1424.

5.6.12. D-Tryptophan-D-glutamic acid (**1l**)

Compound **1l** was prepared from *N*-carbobenzoxyl-D-tryptophan-D-glutamate dibenzylester (**5l**) using a procedure similar to that described for the preparation of **1a** as a white solid, yield 100%. Mp 170–172 °C; $[\alpha]_D^{20}$ −30.4 (*c* 0.5, water); ^{1}H NMR (CD_3OD): $\delta = 7.69$ (d, 1H), 7.38 (d, 1H), 7.22 (s, 1H), 7.11 (t, 1H), 4.33 (m, 1H), 4.19 (q, 1H), 3.47 (dd, 1H), 3.20 (q, 1H), 2.34 (t, 2H), 2.18 (m, 1H), 2.00 (m, 1H); ESI-MS *m/z* 334, $[M + H]^+$; HRMS (ESI) *m/z* calcd $C_{16}H_{20}N_3O_5$ $[M + H]^+$ 334.1403, found 334.1419.

5.7. N-Carbobenzoxyl-L-tryptophan p-nitrophenyl ester (**6**)

To a stirred solution of *N*-carbobenzoxyl-L-tryptophan (3 g, 8.9 mmol) and *p*-nitrophenol (1.26 g, 9.1 mmol) in THF (30 mL) was added *N*,*N*′-dicyclohexylcarbodiimide (DCC, 1.8 g, 8.9 mmol). The solution was kept at ambient temperature for 4 h and then filtered. The filtrate was condensed under reduced pressure below 45 °C and purified by flash chromatography with ethyl acetate/petrol ether (1/2 v/v), and 3.5 g of compound **6** was obtained (85%). Mp 105–107 °C. ^{1}H NMR ($CDCl_3$): $\delta = 8.19$ (s, 1H), 8.16 (br, 2H), 7.56 (d, 1H), 7.40 (d, 1H), 7.34 (br, 5H), 7.23 (t, 1H), 7.11 (m, 2H), 6.97 (d, 2H), 5.14 (s, 2H), 3.45 (m, 2H).

5.8. General procedures for preparation of **8a–d** using N-carbobenzoxyl-L-tryptophan-L-arginine (**8c**) as an example

5.8.1. N-Carbobenzoxyl-L-tryptophan-L-arginine (**8c**)

Unprotected arginine (0.23 g, 1.3 mmol) was dissolved in water (5 mL) at 45 °C, then the solution was cooled to room temperature and triethylamine (0.26 g, 2.6 mmol) was added. Then compound **6** (0.46 g, 1 mmol) in THF (5 mL) was added dropwise and stirred overnight. After evaporation, the oil was purified by flash chromatography (ethyl acetate/petrol ether = 1/2, v/v, then CH_2Cl_2/methane = 5/1, v/v) yielding a white solid **8c** (0.8 g, yield 94%). Mp 89–90 °C. ^{1}H NMR (CD_3OD): $\delta = 7.60$ (d, 1H), 7.28 (m, 5H), 7.07 (m, 2H), 6.97 (t, 1H), 5.00 (d, 2H), 4.43 (q, 1H), 4.23 (t, 1H), 3.30 (m, 3H), 3.10 (m, 3H), 1.82 (m, 1H), 1.68 (m, 1H), 1.50 (m, 2H).

5.8.2. N-Carbobenzoxyl-L-tryptophan-L-glutamine (**8a**)

Compound **8a** was prepared from *N*-carbobenzoxyl-L-tryptophan *p*-nitrophenyl ester (**6**) and L-glutamine using a procedure similar to that described for the preparation of **8c** as a white solid, yield 99%. Mp 148–149 °C; ^{1}H NMR (CD_3OD): $\delta = 7.59$ (d, 1H), 7.2–7.35 (m, 6H), 7.09 (s, 1H), 7.06 (t, 1H), 6.98 (t, 1H), 4.97 (s, 2H), 4.46 (q, 1H), 4.25 (m, 1H), 3.34 (s, 1H), 3.10 (q, 1H), 3.15 (m, 3H), 1.92 (m, 1H).

5.8.3. N-Carbobenzoxyl-L-tryptophan-L-asparagine (**8b**)

Compound **8b** was prepared from *N*-carbobenzoxyl-L-tryptophan *p*-nitrophenyl ester (**6**) and L-asparagine using a procedure similar to that described for the preparation of **8c** as a white solid, yield 98%. Mp 140–141 °C; ^{1}H NMR (CD_3OD): $\delta = 7.56$ (d, 2H), 7.25 (m, 7H), 7.07 (s, 1H), 7.05 (t, 1H), 6.95 (t, 1H), 4.58 (t, 1H), 4.48 (t, 1H), 3.12 (m, 2H), 2.70 (m, 2H).

*5.8.4. N-Carbobenzoxyl-L-tryptophan-L-leucine (**8d**)*

Compound **8d** was prepared from *N*-carbobenzoxyl-L-tryptophan *p*-nitrophenyl ester (**6**) and L-leucine using a procedure similar to that described for the preparation of **8c** as a white solid, yield 90%. Mp 120–121 °C; ^{1}H NMR (CD_3OD): $\delta = 8.167$ (s, 1H), 7.69 (d, 1H), 7.34 (m, 6H), 7.13 (t, 1H), 7.11 (t, 1H), 7.08 (s, 1H), 6.08 (d, 1H), 5.52 (d, 1H), 5.11 (s, 2H), 4.51 (m, 2H), 3.31 (dd, 1H), 3.15 (q, 1H), 1.40 (m, 3H), 0.82 (t, 6H).

*5.9. General procedures for preparation of **9a–d** using L-tryptophan-L-arginine (**9c**) as an example*

*5.9.1. L-Tryptophan-L-arginine (**9c**)*

A solution of **8c** (0.59 g, 1.2 mmol) and TIPS (150 μL) in 80% methanol–water (15 mL) was hydrogenated in the presence of 0.05 g of 10% palladium on charcoal for 8 h. After filtration, the solution was evaporated and dissolved in water (20 mL), and washed with ethyl acetate (50 mL × 3). The water portion was followed by lyophilization to form a white solid **9c** (0.4 g, 100%). Mp 94–95 °C; $[\alpha]_D^{20}$ +22.0 (*c* 0.5, water); ^{1}H NMR (DMSO-d_6): $\delta = 10.93$ (s, 1H), 9.16 (d, 1H), 8.13 (d, 1H), 7.54 (d, 1H), 7.33 (d, 1H), 7.20 (s, 1H), 7.05 (t, 1H), 6.96 (t, 1H), 3.91 (t, 1H), 3.43 (m, 2H), 3.01 (s, 2H), 2.72 (q, 1H), 1.62 (m, 2H), 1.55 (d, 2H); ESI-MS *m/z* 361, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{17}H_{25}N_6O_3$ $[M+H]^+$ 361.1988, found 361.1978.

*5.9.2. L-Tryptophan-L-glutamine (**9a**)*

Compound **9a** was prepared from *N*-carbobenzoxyl-L-tryptophan-L-glutamine (**8a**) using a procedure similar to that described for the preparation of **9c** as a white solid, yield 99%. Mp 165–167 °C; $[\alpha]_D^{20}$ +30.1 (*c* 0.5, water); ^{1}H NMR (CD_3OD): $\delta = 7.58$ (d, 1H), 7.35 (d, 1H), 7.22 (t, 1H), 6.99 (t, 1H), 4.46 (t, 1H), 4.26 (m, 1H), 3.20 (t, 1H), 2.23 (m, 3H), 1.90 (m, 1H); ESI-MS *m/z* 333, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{16}H_{21}N_4O_4$ $[M+H]^+$ 333.1563, found 333.1577.

*5.9.3. L-Tryptophan-L-asparagine (**9b**)*

Compound **9b** was prepared from *N*-carbobenzoxyl-L-tryptophan-L-asparagine (**8b**) using a procedure similar to that described for the preparation of **9c** as a white solid, 97%. Mp 143–144 °C; $[\alpha]_D^{20}$ −2.1 (*c* 0.5, water); ^{1}H NMR (DMSO-d_6): $\delta = 7.61$ (d, 1H), 7.34 (d, 1H), 7.22 (s, 1H), 6.99 (m, 3H), 4.38 (s, 1H), 3.63 (m, 2H), 3.20 (dd, 2H), 2.81 (q, 2H); ESI-MS *m/z* 319, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{15}H_{19}N_3O_5$ $[M+H]^+$ 319.1416, found 319.1406.

*5.9.4. L-Tryptophan-L-leucine (**9d**)*

Compound **9d** was prepared from *N*-carbobenzoxyl-L-tryptophan-L-leucine (**8d**) using a procedure similar to that described for the preparation of **9c** as a white solid, yield 95%. Mp 122–124 °C; $[\alpha]_D^{20}$ +15.1 (*c* 0.5, water); ^{1}H NMR (DMSO-d_6): $\delta = 10.95$ (d, 1H), 8.73 (m, 1H), 7.60 (d, 1H), 7.31 (d, 1H), 7.19 (s, 1H), 7.03 (t, 1H), 6.94 (t, 1H), 4.16 (q, 1H), 3.67 (q, 1H), 3.15 (dd, 1H), 2.85 (q, 1H), 1.48 (m, 3H), 0.82 (t, 6H); ESI-MS *m/z* 318, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{17}H_{24}N_3O_3$ $[M+H]^+$ 318.1818, found 318.1807.

*5.10. General procedures for preparation of **12a–t** using **12a** as an example*

*5.10.1. N-tert-Butoxycarbonyl-L-tryptophan-L-aspartate dibenzylester (**12a**)*

To a mixture of *N-tert*-butoxycarbonyl-L-tryptophan (0.46 g, 1.5 mmol), L-dibenzylester aspartate *p*-toluenesulfonate (**3b**, 0.73 g, 1.5 mmol), 1-hydroxybenzotriazole (0.2 g, 1.5 mmol) and triethylamine (1.4 mL, 10 mmol) in 5% DMF in CH_2Cl_2 (20 mL), 1-ethyl-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI, 0.4 g, 2 mmol) was added in portions for 5 min. The solution was stirred overnight at rt. The solution was washed, dried, filtered and condensed *in vacuo* to form a yellow oil. The oil can be purified by flash chromatography with petroleum ether/ethyl acetate (2/1, v/v) to afford a white powder **12a** (0.89 g, 99%). Mp 60–61 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.55$ (d, 1H), 7.20–7.39 (br s, 11H), 7.07 (m, 2H), 7.00 (t, 1H), 5.08 (s, 2H), 5.03 (s, 2H), 4.83 (m, 1H), 4.36 (m, 1H), 3.18 (dd, 1H), 3.00 (m, 1H), 2.80 (t, 2H), 1.34 (s, 9H).

*5.10.2. N-tert-Butoxycarbonyl-L-tryptophan-L-glutamate dimethylester (**12b**)*

Compound **12b** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan and L-glutamate dimethylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 92%. Mp 105–107 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.19$ (s, 1H), 7.63 (d, 1H), 7.35 (d, 1H), 7.17 (t, 1H), 7.11 (t, 1H), 7.09 (s, 1H), 6.44 (br, 1H), 5.12 (br, 1H), 4.51 (m, 1H), 4.44 (m, 1H), 3.64 (s, 3H), 3.62 (s, 3H), 3.33 (dd, 1H), 3.15 (q, 1H), 2.12 (m, 3H), 1.87 (m, 1H).

*5.10.3. N-tert-Butoxycarbonyl-L-tryptophan-L-aspartate dimethylester (**12c**)*

Compound **12c** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan and L-aspartate dimethylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 80%. Mp 76–78 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.21$ (br, 1H), 7.63 (d, 1H), 7.34 (d, 1H), 7.16 (t, 1H), 7.10 (t, 1H), 7.08 (s, 1H), 6.78 (d, 1H), 5.12 (br, 1H), 4.72 (s, 1H), 4.45 (br, 1H), 3.66 (s, 3H), 3.57 (s, 3H), 3.29 (dd, 1H), 3.18 (q, 1H), 2.88 (m, 2H), 2.75 (dd, 1H), 1.42 (s, 9H).

*5.10.4. N-tert-Butoxycarbonyl-L-tryptophan-L-2-aminoadipate dimethylester (**12d**)*

Compound **12d** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan and L-2-aminoadipate dimethylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 90%. Mp 130–131 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.61$ (d, 1H), 7.33 (d, 2H), 7.08 (m, 3H), 4.45 (m, 1H), 4.07 (m, 1H), 3.65 (s, 3H), 3.62 (s, 3H), 3.33 (dd, 1H), 3.21 (q, 1H), 2.21 (m, 2H), 1.62 (m, 2H), 1.52 (m, 2H), 1.48 (s, 9H).

*5.10.5. N-tert-Butoxycarbonyl-L-tryptophan-L-leucine benzylester (**12e**)*

Compound **12e** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan and L-leucine benzylester using a procedure similar to that described for the preparation of **12a** as a white solid, yield 95%. Mp 90–91 °C; $[\alpha]_D^{20}$ −1.2 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 7.65$ (d, 1H), 7.34 (d, 1H), 7.16 (m, 6H), 7.07 (m, 2H), 5.12 (s, 2H), 4.58 (m, 1H), 3.84 (dd, 1H), 3.40 (dd, 1H), 2.95 (q, 1H), 1.55 (m, 3H), 1.48 (s, 9H), 0.90 (s, 3H), 0.88 (s, 3H).

*5.10.6. N-tert-Butoxycarbonyl-L-tryptophan-L-leucine methylester (**12f**)*

Compound **12f** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan and L-leucine methylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 90%. Mp 68–70 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.35$ (d, 1H), 7.65 (d, 1H), 7.33 (d, 1H), 7.16 (t, 1H), 7.07 (m, 2H), 4.45 (m, 1H), 3.75 (dd, 1H), 3.34 (dd, 1H), 2.95 (q, 1H), 1.55 (m, 3H), 1.48 (s, 9H), 1.20 (s, 3H), 1.17 (s, 3H).

*5.10.7. N-tert-Butoxycarbonyl-L-2-naphthylalanine-L-glutamate dibenzylester (**12g**)*

Compound **12g** was prepared from *N-tert*-butoxycarbonyl-L-2-naphthylalanine and L-glutamate dibenzylester *p*-toluenesulfonate (**3a**) using a procedure similar to that described for the preparation of **12a** as a white solid, yield 98%. Mp 140–141 °C; ^{1}H NMR (CD_3OD): $\delta = 7.73$ (m, 3H), 7.65 (s, 1H), 7.27–7.40 (m, 13H), 5.11 (d, 2H), 5.02 (s, 2H), 4.41 (m, 1H), 3.17 (dd, 1H), 2.97 (q, 1H), 2.05 (m, 3H), 1.80 (m, 1H), 1.28 (s, 9H).

*5.10.8. N-tert-Butoxycarbonyl-L-tryptophan-D-glutamate dimethylester (**12h**)*

Compound **12h** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan and D-glutamate dimethylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 90%. ^{1}H NMR ($CDCl_3$): $\delta = 7.62$ (d, 1H), 7.35 (d, 1H), 7.16 (t, 1H), 7.08 (t, 1H), 7.05 (s, 1H), 4.43 (m, 1H), 4.22 (m, 1H), 3.23 (s, 3H), 3.18 (s, 3H), 3.08 (dd, 1H), 3.01 (q, 1H), 2.11 (m, 3H), 1.90 (m, 1H).

*5.10.9. N-tert-Butoxycarbonyl-L-phenylalanine-L-aspartate dibenzylester (**12i**)*

Compound **12i** was prepared from *N-tert*-butoxycarbonyl-L-phenylalanine and L-aspartate dibenzylester *p*-toluenesulfonate (**3b**) using a procedure similar to that described for the preparation of **12a** as a white solid, yield 98%. ^{1}H NMR ($CDCl_3$): $\delta = 7.84$ (s, 1H), 7.60 (d, 1H), 6.77–7.36 (m, 13H), 6.96 (s, 1H), 6.75 (d, 1H), 5.06 (s, 2H), 4.95 (d, 2H), 4.76 (m, 1H), 4.43 (br, 1H), 3.48 (dd, 1H), 3.17 (q, 1H), 2.99 (dd, 1H), 2.81 (m, 1H), 1.41 (s, 9H).

*5.10.10. N-tert-Butoxycarbonyl-L-phenylalanine-L-glutamate dimethylester (**12j**)*

Compound **12j** was prepared from *N-tert*-butoxycarbonyl-L-phenylalanine and L-glutamate dimethylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 99%. ^{1}H NMR ($CDCl_3$): $\delta = 7.23$ (m, 5H), 4.68 (t, 1H), 3.64 (s, 3H), 3.58 (s, 3H), 3.52 (m, 1H), 2.90 (m, 2H), 2.66 (q, 2H), 1.42 (s, 9H).

*5.10.11. N-tert-Butoxycarbonyl-L-2-naphthylalanine-L-glutamate dimethylester (**12k**)*

Compound **12k** was prepared from *N-tert*-butoxycarbonyl-L-2-naphthylalanine and L-glutamate dimethylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 99%. Mp 136–137 °C; ^{1}H NMR (CD_3OD): $\delta = 7.76$–7.81 (m, 3H), 7.64 (m, 1H), 7.45 (m, 2H), 7.35 (dd, 1H), 6.66 (br, 1H), 5.02 (br, 1H), 4.50 (m, 2H), 3.67 (s, 3H), 3.60 (s, 3H), 3.22 (d, 2H), 2.08 (m, 3H), 1.81 (m, 1H), 1.38 (s, 9H).

*5.10.12. N-tert-Butoxycarbonyl-L-tryptophan-D-glutamate dibenzylester (**12l**)*

Compound **12l** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan and L-glutamate dibenzylester *p*-toluenesulfonate (**3a**) using a procedure similar to that described for the preparation of **12a** as a white solid, yield 85%. Mp 123–124 °C; ^{1}H NMR (CD_3OD): $\delta = 7.54$ (d, 1H), 7.24–7.34 (m, 11H), 7.04 (m, 3H), 5.10 (d, 2H), 5.06 (s, 2H), 4.35 (m, 2H), 3.14 (dd, 1H), 3.02 (q, 1H), 2.01 (m, 3H), 1.78 (m, 1H).

*5.10.13. N-tert-Butoxycarbonyl-L-phenylalanine-L-glutamate dibenzylester (**12m**)*

Compound **12m** was prepared from *N-tert*-butoxycarbonyl-L-phenylalanine and L-glutamate dibenzylester *p*-toluenesulfonate (**3a**) using a procedure similar to that described for the preparation of **12a** as a white solid, yield 95%. Mp 100–101 °C; ^{1}H NMR (CD_3OD): $\delta = 7.35$ (m, 10H), 7.23 (m, 5H), 6.55 (d, 1H), 5.15 (d, 2H), 5.10 (s, 2H), 4.62 (m, 1H), 4.34 (d, 1H), 3.05 (m, 2H), 2.30 (m, 3H), 1.98 (m, 1H), 1.63 (s, 2H), 1.40 (s, 9H).

*5.10.14. N-tert-Butoxycarbonyl-L-2-naphthylalanine-L-leucine methylester (**12n**)*

Compound **12n** was prepared from *N-tert*-butoxycarbonyl-L-2-naphthylalanine and L-leucine methylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 99%. Mp 41–42 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.79$ (m, 3H), 7.64 (s, 1H), 7.47 (m, 2H), 7.37 (d, 1H), 6.12 (d, 1H), 5.07 (br, 1H), 4.48 (br, 2H), 3.67 (s, 3H), 3.23 (m, 2H), 1.74 (s, 1H), 1.39 (s, 9H), 0.70 (t, 6H).

*5.10.15. N-tert-Butoxycarbonyl-D-phenylalanine-L-2-aminoadipate dimethylester (**12o**)*

Compound **12o** was prepared from *N-tert*-butoxycarbonyl-D-phenylalanine and L-2-aminoadipate dimethylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 99%. Mp

85–86 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.19$–7.31 (m, 5H), 6.38 (d, 1H), 5.00 (br, 1H), 4.51 (q, 1H), 4.37 (q, 1H), 3.72 (s, 3H), 3.67 (s, 3H), 3.05 (d, 2H), 2.25 (t, 2H), 1.71 (m, 1H), 1.58 (m, 1H), 1.41 (s, 9H).

5.10.16. N-tert-Butoxycarbonyl-D-phenylalanine-L-leucine methylester (**12p**)

Compound **12p** was prepared from *N-tert*-butoxycarbonyl-D-phenylalanine and L-leucine methylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 98%. Mp 128–129 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.19$–7.33 (m, 5H), 6.15 (d, 1H), 4.99 (br, 1H), 4.52 (m, 1H), 4.37 (m, 1H), 3.70 (s, 3H), 3.06 (d, 1H), 1.67 (m, 1H), 1.39 (s, 9H), 0.85 (t, 6H).

5.10.17. N-tert-Butoxycarbonyl-D-phenylalanine-L-aspartate dimethylester (**12q**)

Compound **12q** was prepared from *N-tert*-butoxycarbonyl-D-phenylalanine and L-aspartate dimethylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 96%. Mp 100–101 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.19$–7.32 (m, 5H), 4.88 (m, 1H), 3.65 (s, 3H), 3.62 (s, 3H), 3.59 (m, 1H), 2.92 (m, 2H), 2.68 (m, 2H), 1.45 (s, 9H).

5.10.18. N-tert-Butoxycarbonyl-D-phenylalanine-L-glutamate dimethylester (**12r**)

Compound **12r** was prepared from *N-tert*-butoxycarbonyl-D-phenylalanine and L-glutamate dimethylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 95%. Mp 95–96 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.18$–7.31 (m, 5H), 6.53 (d, 1H), 4.96 (br, 1H), 4.55 (m, 1H), 4.33 (q, 1H), 3.70 (s, 3H), 3.06 (t, 2H), 2.22 (m, 2H), 2.15 (m, 1H), 1.90 (m, 1H), 1.41 (s, 9H).

5.10.19. O-Methyl-N-tert-butoxycarbonyl-L-tyrosine-L-glutamate dimethylester (**12s**)

Compound **12s** was prepared from *O*-methyl-*N-tert*-butoxycarbonyl-L-tyrosine and L-glutamate dimethylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 98%. Mp 99–100 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.10$ (d, 2H), 6.83 (d, 2H), 6.51 (d, 1H), 4.94 (br, 1H), 4.56 (m, 1H), 4.29 (q, 1H), 3.78 (s, 3H), 3.71 (s, 3H), 3.66 (s, 3H), 2.97 (m, 2H), 2.13–2.34 (m, 3H), 1.94 (m, 1H), 1.37 (s, 9H).

5.10.20. N-tert-Butoxycarbonyl-L-phenylalanine-L-alanine methylester (**12t**)

Compound **12t** was prepared from *N-tert*-butoxycarbonyl-L-phenylalanine and L-alanine methylester hydrochloride using a procedure similar to that described for the preparation of **12a** as a white solid, yield 95%. Mp 99–101 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.18$–7.31 (m, 5H), 6.47 (d, 1H), 5.01 (br, 1H), 4.51 (q, 1H), 4.36 (br, 1H), 3.70 (s, 3H), 3.06 (t, 2H), 1.42 (s, 9H), 1.33 (d, 3H).

5.11. General procedures for preparation of **13a–t** using **13a** as an example

5.11.1. L-Tryptophan-L-aspartate dibenzylester (**13a**)

To a solution of **12a** (0.5 g, 0.8 mmol) in anhydrous CH_2Cl_2 (10 mL) and TIPS (0.1 mL) in ice bath, TFA (0.25 mL) was added. After 4 h the solution was allowed to gradually reach ambient temperature and stirred for another 8 h. The solvent and TFA were removed by reduced pressure below 30 °C using additional amounts of CH_2Cl_2 to help complete evaporation of TFA. The dark oil was purified by chromatography with CH_2Cl_2/MeOH (20/1, v/v) to form a white solid **13a** (0.3 g, 82%). Mp 88–89 °C; $[\alpha]_D^{20}$ −21.2 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) $\delta = 7.59$ (t, 2H), 7.29 (m, 8H), 7.18 (m, 4H), 6.44 (s, 1H), 6.22 (s, 1H), 5.05 (s, 2H), 4.97 (dd, H), 4.70 (s, 2H), 3.33 (m, 2H), 2.78 (m, 2H); ESI-MS *m/z* 500, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{29}H_{30}N_3O_5$ $[M+H]^+$ 500.2182, found 500.2185.

5.11.2. L-Tryptophan-L-glutamate dimethylester (**13b**)

Compound **13b** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan-L-glutamate dimethylester (**12b**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 90%. Mp 110–111 °C; $[\alpha]_D^{20}$ −12.7 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 8.33$ (br, 1H), 7.90 (d, 1H), 7.67 (d, 1H), 7.38 (d, 1H), 7.20 (t, 1H), 7.12 (m, 2H), 4.60 (m, 1H), 3.83 (q, 1H), 3.77 (s, 3H), 3.67 (s, 3H), 3.35 (dd, 1H), 3.06 (q, 1H), 2.30 (m, 4H); ESI-MS *m/z* 362, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{18}H_{24}N_3O_5$ $[M+H]^+$ 362.1700, found 362.1716.

5.11.3. L-Tryptophan-L-aspartate dimethylester (**13c**)

Compound **13c** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan-L-aspartate dimethylester (**12c**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 65%. Mp 231–233 °C; $[\alpha]_D^{20}$ −42.8 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 8.15$ (1H), 7.64 (d, 1H), 7.34 (d, 1H), 7.16 (t, 1H), 7.11 (t, 1H), 7.09 (s, 1H), 6.75 (d, 1H), 5.18 (m, 1H), 4.73 (m, 1H), 4.45 (m, 1H), 3.67 (s, 3H), 3.58 (s, 3H), 3.29 (dd, 1H), 3.20 (q, 1H), 2.93 (m, 1H), 2.76 (m, 1H), 2.68 (dd, 1H); ESI-MS *m/z* 348, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{17}H_{22}N_3O_5$ $[M+H]^+$ 348.1559, found 348.1548.

5.11.4. L-Tryptophan-L-2-aminoadipate dimethylester (**13d**)

Compound **13d** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan-L-2-aminoadipate dimethylester (**12d**) using a procedure similar to that described for the preparation of **13a** as a yellow oil, yield 88%. Oil; $[\alpha]_D^{20}$ −4.6 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 8.78$ (br, 1H), 7.95 (m, 1H), 7.60 (d, 1H), 7.38 (d, 1H), 7.15 (m, 2H), 7.06 (t, 1H), 4.44 (m, 1H), 4.05 (m, 1H), 3.67 (s, 3H), 3.61 (s, 3H), 3.26 (dd, 1H), 3.21 (q, 1H), 2.22 (m, 2H), 1.74 (m, 1H), 1.62 (m, 1H), 1.51 (m, 2H), 1.24 (s, 1H); ESI-MS *m/z* 376, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{19}H_{26}N_3O_5$ $[M+H]^+$ 376.1872, found 376.1878.

*5.11.5. L-Tryptophan-L-leucine benzylester (**13e**)*

Compound **13e** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan-L-leucine benzylester (**12e**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 93%. Mp 78–80 °C; $[\alpha]_D^{20}$ +5.4 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 7.66$ (d, 1H), 7.34 (d, 1H), 7.16 (m, 6H), 7.10 (s, 1H), 7.07 (t, 1H), 5.12 (s, 2H), 4.58 (m, 1H), 3.83 (m, 1H), 3.27 (dd, 1H), 2.95 (q, 1H), 1.56 (m, 1H), 1.50 (m, 2H), 0.87 (s, 3H), 0.85 (s, 3H); ESI-MS *m/z* 408, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{24}H_{30}N_3O_3$ $[M+H]^+$ 408.2287, found 408.2301.

*5.11.6. L-Tryptophan-L-leucine methylester (**13f**)*

Compound **13f** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan-L-leucine methylester (**12f**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 76%. Mp > 250 °C; $[\alpha]_D^{20}$ −1.2 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 8.40$ (br, 1H), 7.66 (d, 1H), 7.43 (d, 1H), 7.16 (t, 1H), 7.07 (t, 1H), 7.03 (s, 1H), 6.08 (d, 1H), 4.40 (m, 1H), 3.7 (dd, 1H), 3.38 (dd, 1H), 2.95 (q, 1H), 1.55 (m, 1H), 1.52 (m, 2H), 1.20 (s, 3H), 1.17 (s, 3H); ESI-MS *m/z* 332, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{18}H_{26}N_3O_3$ $[M+H]^+$ 332.1974, found 332.1980.

*5.11.7. L-2-Naphthylalanine-L-glutamate dibenzylester (**13g**)*

Compound **13g** was prepared from *N-tert*-butoxycarbonyl-L-2-naphthylalanine-L-glutamate dibenzylester (**12g**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 92%. Mp 100–102 °C; $[\alpha]_D^{20}$ −36.4 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD): $\delta = 7.99$ (s, 1H), 7.69–7.82 (m, 4H), 7.27–7.44 (m, 13H), 5.48 (s, 1H), 5.13 (s, 2H), 5.01 (s, 2H), 4.43 (dd, 1H), 4.10 (m, 1H), 3.21 (m, 2H), 1.90 (m, 3H), 1.72 (m, 1H); ESI-MS *m/z* 525, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{32}H_{33}N_2O_5$ $[M+H]^+$ 525.2390, found 525.2377.

*5.11.8. L-Tryptophan-D-glutamate dimethylester (**13h**)*

Compound **13h** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan-D-glutamate dimethylester (**12h**) using a procedure similar to that described for the preparation of **13a** as a white solid. Mp 120–121 °C; $[\alpha]_D^{20}$ −2.5 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 8.32$ (br, 1H), 7.70 (br, 1H), 7.67 (d, 1H), 7.40 (d, 1H), 7.25 (t, 1H), 7.18 (t, 1H), 7.10 (s, 1H), 4.44 (m, 1H), 3.78 (q, 1H), 3.65 (s, 3H), 3.57 (s, 3H), 3.35 (dd, 1H), 3.21 (q, 1H), 2.30 (m, 4H); ESI-MS *m/z* 362, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{18}H_{24}N_3O_5$ $[M+H]^+$ 362.1700, found 362.1706.

*5.11.9. L-Phenylalanine-L-aspartate dibenzylester (**13i**)*

Compound **13i** was prepared from *N-tert*-butoxycarbonyl-L-phenylalanine-L-aspartate dibenzylester (**12i**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 92%. Mp 136–137 °C; $[\alpha]_D^{20}$ +1.7 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 7.63$ (d, 1H), 7.12–7.32 (m, 15H), 5.03 (s, 2H), 4.92 (d, 2H), 4.74 (m, 1H), 4.30 (m, 1H), 3.12 (d, 2H), 2.84 (m, 2H); ESI-MS *m/z* 461, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{27}H_{29}N_2O_5$ $[M+H]^+$ 461.2077, found 461.2089.

*5.11.10. L-Phenylalanine-L-glutamate dimethylester (**13j**)*

Compound **13j** was prepared from *N-tert*-butoxycarbonyl-L-phenylalanine-L-glutamate dimethylester (**12j**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 94%. Mp 225–226 °C; $[\alpha]_D^{20}$ +3.4 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 7.24$ (m, 5H), 4.65 (t, 1H), 3.66 (s, 3H), 3.63 (s, 3H), 3.52 (m, 1H), 2.88 (m, 2H), 2.60 (m, 2H); ESI-MS *m/z* 309, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{15}H_{21}N_2O_5$ $[M+H]^+$ 309.1450, found 309.1438.

*5.11.11. L-2-Naphthylalanine-L-glutamate dimethylester (**13k**)*

Compound **13k** was prepared from *N-tert*-butoxycarbonyl-L-2-naphthylalanine-L-glutamate dimethylester (**12k**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 97%. Mp 89–90 °C; $[\alpha]_D^{20}$ −45.9 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD): $\delta = 7.77$ (m, 3H), 7.66 (m, 1H), 7.34 (m, 2H), 7.23 (dd, 1H), 6.50 (d, 1H), 5.02 (m, 1H), 4.52 (m, 2H), 3.57 (s, 3H), 3.50 (s, 3H), 3.12 (d, 2H), 2.18 (m, 3H), 1.90 (m, 1H); ESI-MS *m/z* 373, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{20}H_{25}N_2O_5$ $[M+H]^+$ 373.1763, found 373.1763.

*5.11.12. L-Tryptophan-D-glutamate dibenzylester (**13l**)*

Compound **13l** was prepared from *N-tert*-butoxycarbonyl-L-tryptophan-D-glutamate dibenzylester (**12l**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 87%. Mp 54–55 °C; $[\alpha]_D^{20}$ +44.3 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 7.54$ (br, 1H), 6.89–7.31 (m, 15H), 4.94 (s, 2H), 4.71 (br, 2H), 4.31 (m, 1H), 3.35 (br, 2H), 2.89 (m, 1H), 1.82 (m, 4H); ESI-MS *m/z* 514, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{30}H_{32}N_3O_5$ $[M+H]^+$ 514.2342, found 514.2356.

*5.11.13. L-Phenylalanine-L-glutamate dibenzylester (**13m**)*

Compound **13m** was prepared from *N-tert*-butoxycarbonyl-L-phenylalanine-L-glutamate dibenzylester (**12m**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 89%. Mp 190–192 °C; $[\alpha]_D^{20}$ −35.7 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD): $\delta = 7.66$ (d, 1H), 7.09–7.41 (m, 15H), 5.13 (s, 2H), 5.01 (d, 2H), 4.76 (m, 1H), 4.44 (m, 1H), 3.12 (m, 2H), 2.03 (m, 3H), 1.78 (m, 1H); ESI-MS *m/z* 475, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{28}H_{31}N_2O_5$ $[M+H]^+$ 475.2234, found 475.2248.

*5.11.14. L-2-Naphthylalanine-L-leucine methylester (**13n**)*

Compound **13n** was prepared from *N-tert*-butoxycarbonyl-L-2-naphthylalanine-L-leucine methylester (**12n**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 94%. Mp 94–96 °C; $[\alpha]_D^{20}$ −70.0 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 8.21$ (br, 1H), 7.77 (m, 4H), 7.52 (d, 1H), 7.42 (q, 2H), 4.70 (br, 1H), 4.38 (m, 1H), 3.43 (s, 3H), 1.35 (m, 2H), 1.29 (s, 1H), 1.12 (m, 1H), 0.85 (m, 1H), 0.65 (q, 6H); ESI-MS *m/z* 343, $[M+H]^+$; HRMS

(ESI) *m/z* calcd $C_{20}H_{27}N_2O_3$ $[M+H]^+$ 343.2022, found 343.2015.

*5.11.15. D-Phenylalanine-L-2-aminoadipate dimethylester (**13o**)*

Compound **13o** was prepared from *N-tert*-butoxycarbonyl-D-phenylalanine-L-2-aminoadipate dimethylester (**12o**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 96%. Mp 218–219 °C; $[\alpha]_D^{20}$ −26.5 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 7.83$ (br, 1H), 7.23–7.33 (m, 5H), 4.55 (q, 1H), 3.80 (m, 1H), 3.72 (s, 3H), 3.65 (s, 3H), 3.28 (dd, 1H), 2.78 (q, 1H), 2.29 (m, 3H), 1.83 (m, 1H), 1.61 (m, 2H); ESI-MS *m/z* 337, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{17}H_{25}N_2O_5$ $[M+H]^+$ 337.1763, found 337.1795.

*5.11.16. D-Phenylalanine-L-leucine methylester (**13p**)*

Compound **13p** was prepared from *N-tert*-butoxycarbonyl-D-phenylalanine-L-leucine methylester (**12p**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 95%. Mp 82–84 °C; $[\alpha]_D^{20}$ −81.0 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD): $\delta = 7.22$–7.34 (m, 5H), 4.32 (q, 1H), 3.88 (t, 1H), 3.69 (s, 3H), 3.01 (dd, 2H), 1.65 (m, 1H), 1.37 (s, 9H), 0.85 (t, 6H); ESI-MS *m/z* 293, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{16}H_{25}N_2O_3$ $[M+H]^+$ 293.1865, found 293.1855.

*5.11.17. D-Phenylalanine-L-aspartate dimethylester (**13q**)*

Compound **13q** was prepared from *N-tert*-butoxycarbonyl-D-phenylalanine-L-aspartate dimethylester (**12q**) using a procedure similar to that described for the preparation of **13a** as a white solid. Mp 59–60 °C; $[\alpha]_D^{20}$ −19.8 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD): $\delta = 7.19$–7.27 (m, 5H), 4.75 (t, 1H), 3.70 (s, 3H), 3.64 (s, 3H), 3.59 (t, 1H), 2.92 (q, 1H), 2.81 (q, 1H), 2.68 (dd, 2H); EI-MS *m/z* 309 $(M+1)$, 120 (100%); HRMS (EI) *m/z* calcd $C_{15}H_{21}N_2O_5$ $(M+1)$ 309.1445, found 309.1450.

*5.11.18. D-Phenylalanine-L-glutamate dimethylester (**13r**)*

Compound **13r** was prepared from *N-tert*-butoxycarbonyl-D-phenylalanine-L-glutamate dimethylester (**12r**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 93%. Mp 121–122 °C; $[\alpha]_D^{20}$ −8.3 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 7.20$–7.33 (m, 5H), 6.55 (d, 1H), 5.01 (br, 1H), 4.57 (m, 1H), 4.37 (m, 1H), 3.73 (s, 3H), 3.68 (s, 3H), 3.07 (d, 2H), 2.16 (m, 2H), 1.86 (m, 1H); ESI-MS *m/z* 323, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{16}H_{23}N_2O_5$ $[M+H]^+$ 323.1607, found 323.1601.

*5.11.19. O-Methyl-L-tyrosine-L-glutamate dimethylester (**13s**)*

Compound **13s** was prepared from *O*-methyl-*N-tert*-butoxycarbonyl-L-tyrosine-L-glutamate dimethylester using a procedure similar to that described for the preparation of **13a** as a white solid, yield 91%. Mp 253–254 °C; $[\alpha]_D^{20}$ −0.8 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD): $\delta = 7.12$ (d, 2H), 6.85 (d, 2H), 4.41 (q, 1H), 3.76 (s, 3H), 3.69 (s, 3H), 3.61 (s, 3H), 2.95 (dd, 1H), 2.84 (q, 1H), 2.34 (t, 2H), 1.15 (s, 2H), 1.91 (m, 1H); ESI-MS *m/z* 353, $[M+H]^+$; HRMS (ESI) *m/z* calcd $C_{17}H_{25}N_2O_6$ $[M+H]^+$ 353.1713, found 353.1705.

*5.11.20. L-Phenylalanine-L-alanine methylester (**13t**)*

Compound **13t** was prepared from *N-tert*-butoxycarbonyl-L-phenylalanine-L-alanine methylester (**12t**) using a procedure similar to that described for the preparation of **13a** as a white solid, yield 93%. Mp > 250 °C; $[\alpha]_D^{20}$ +13.5 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD): $\delta = 7.18$–7.33 (m, 5H), 4.43 (q, 1H), 4.30 (t, 1H), 3.70 (s, 3H), 2.93 (m, 2H), 1.37 (d, 3H); EI-MS *m/z* 250 (M^+), 120 (100%); HRMS (EI) *m/z* calcd $C_{13}H_{18}N_2O_3$ (M^+) 250.1317, found 250.1325.

Acknowledgements

We gratefully acknowledge the generous support from the National Natural Science Foundation of China (Grants 20372069, 29725203 and 20472094), the Key Technologies R&D Program from CAS (Grants 2006BAI01B02 and 2005BA711A01), and the 863 Hi-Tech Program of China (Grants 2006AA020402 and 2006AA020602).

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