International Journal of Peptide Research and Therapeutics
https://doi.org/10.1007/s10989-018-9680-2

Synthesis and Characterization of a Series of Orthogonally Protected L-Carnosine Derivatives

Mohammad H. El-Dakdouki[1] · Nadine Daouk[1] · Hiba Abdallah[1]

Accepted: 30 January 2018

Abstract
L-Carnosine (β-alanyl-L-histidine) is an endogenous dipeptide that has been recognized for its broad spectrum of beneficial biological activities. However, the therapeutic utility of molecule has been hampered by its instability in human plasma (half-life in human serum < 5 min) due to the presence of carnosinase enzyme that catalyzes its hydrolysis into the respective individual amino acids. While a large number of carnosine derivatives have been synthesized to optimize its overall pharmacokinetic profile, reports that provide molecular evidence as to how the dipeptide interacts with its biological target are scarce. Therefore, many questions are yet to be answered concerning the pharmacophoric regions in carnosine and its significance to the molecule's diverse biological activities. In this study, we set out to construct a small library of carnosine analogues that can be used in assessing the influence of the various functional groups of the dipeptide on its important biological properties. Orthogonal protection/deprotection of selected functional groups led to the exposure of amino group at the N-terminus, the carboxyl group at the C-terminus, and the imidazole ring of histidine. To examine the significance of the imidazole group in preventing the aggregation of the β-amyloid plaques, histidine was replaced by phenylalanine and a series of β-Ala-Phe analogues was generated. To study the influence of the length of the carbon chain in β-Ala on the β-amyloid aggregation, a series of Gly-His analogues was synthesized. A series of Gly-Phe was also constructed and will be used as negative control in future β-amyloid plaque assembly experiments. The synthesized carnosine derivatives were characterized by NMR (proton, carbon, and ^{1}H–^{1}H COSY), and mass spectroscopy.

Keywords L-Carnosine · Orthogonal deprotection · β-Alanine · Histidine · Peptides

Introduction

Given their attractive pharmacological profile and intrinsic properties, peptides represent an excellent starting point for the design of novel therapeutics, and their specificity translated into excellent safety, tolerability, and efficacy profiles in humans (Sachdeva 2017). Peptides are characterized by predictable metabolism, shorter time to market, lower attrition rates, lower production costs, and standard synthetic protocols (Fosgerau and Hoffmann 2015). In fact, the global peptide therapeutics market was valued at USD 18.9 billion in 2013 and is estimated to reach USD 25 billion by 2018 (Craik et al. 2013). Of the simple peptides that has gained much attention as a pharmaceutical agent is L-carnosine (CAR) (Fig. 1), an endogenous dipeptide that is found predominantly in human muscles, heart, liver, brain, kidneys, and other long-lived tissues (Boldyrev 2000; Bauer 2005). CAR is synthesized from β-alanine and L-histidine by the ATP-driven enzyme carnosine synthetase, and is hydrolyzed by specific metal ion-dependent homodimeric dipeptidase called carnosinases in the blood and other tissues (Pegova et al. 2000; Teufel et al. 2003).

CAR possesses activities that suppress age-related dysfunctions (Hipkiss 2009). CAR is also well-documented to exhibit antioxidant properties by scavenging reactive oxygen species (ROS) such as hydroxyl radicals, superoxide and singlet oxygen, as well as reactive carbonyl species (RCS). Moreover, CAR can chelate heavy metals that cause cellular damage, thus protecting macromolecules, such as lipids,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10989-018-9680-2) contains supplementary material, which is available to authorized users.

✉ Mohammad H. El-Dakdouki
m.eldakdouki@bau.edu.lb

[1] Department of Chemistry, Beirut Arab University, Riad El Solh, P.O. Box 11-5020, Beirut 11072809, Lebanon

Published online: 12 February 2018

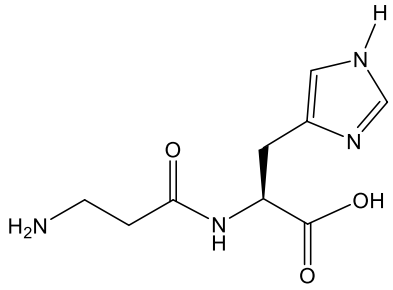


Fig. 1 Chemical structure of L-carnosine (CAR)

proteins and DNA, from damages that result in ageing and age-related disease (Hipkiss et al. 2013). This multifunctional profile of carnosine suggests that the molecule can be associated with neurodegenerative diseases such as Alzheimer's and Parkinson diseases. While there are no current reports of clinical trials concerning CAR's effects towards clinically-defined age-related dysfunction, it is reported that CAR improved cognition in schizophrenics. In senescence-accelerated mice, a diet supplemented with carnosine decreased the signs of ageing and increase mean lifespan by 20% (Boldyrev et al. 1999).

Structure activity relationships suggested that the presence of the β-alanine residue is implicated in the antioxidant properties while the histidine residue has properties including the ability to bind to transition metal ions (Castelletto et al. 2011). Another important role of the histidine residue in carnosine is its ability to inhibit glycation-induced protein crosslinking. In addition, it has been reported that both the amino group of the β-alanyl residue and the imidazole ring of L-histidine act synergistically in trapping cytotoxic α,β-unsaturated aldehydes such as 4-hydroxy-trans-2-nonenal (HNE) (Aldini et al. 2002; Vistoli et al. 2016). Unfortunately, CAR, like other naturally-occurring peptides, is often not directly suitable for use as convenient therapeutics because of its intrinsic weaknesses (Fosgerau and Hoffmann 2015; Hamley 2007). The main limitation for the therapeutic use of CAR is the rapid hydrolysis mostly in human plasma by carnosinase (Hipkiss 2009). A number of strategies have been deployed to overcome this perceived obstacle, essentially through the chemical derivatization, conjugation with several types of potentially beneficial organic molecules, and the synthesis of its structural analogues (Guiotto et al. 2005; Vistoli et al. 2009; Orioli et al. 2011; Bertinaria et al. 2012). In addition, CAR has been conjugated onto different types of nanoparticles for various purposes. However, the conjugation was non-covalent and non-specific, thus potentially hindering functional groups important for the interaction of CAR with its intended biological target (Bellia et al. 2013; Malkar et al. 2015; Durmus et al. 2011; Li et al. 2016; Krpetić et al. 2012; Saada et al. 2011).Therefore, it is highly desirable that L-carnosine is controllably conjugated to the nanoparticles through carefully pre-selected functional groups.

The main goal of this study is to synthesize and characterize a series of L-carnosine analogues with orthogonally protected functional groups. This series can be utilized in future studies aiming at understanding the relationship between CAR's functional groups and its broad spectrum of biological activities. The functional groups of the dipeptide, i.e. the N-terminus amino group, the C-terminal carboxyl group, and the imidazole group of histidine, were protected. Orthogonal deprotection procedures will selectively expose specific functional groups leading to the assembly of a small library of CAR analogues. To examine the significance of the imidazole group, histidine (His) was replaced by phenylalanine (Phe) and a series of β-Ala-Phe analogues was generated. To study the influence of the length of the carbon chain in β-Ala, a series of Gly-His analogues was synthesized. Finally, a series of Gly-Phe derivatives was synthesized and can be used as negative control in future structure–activity relationship studies.

Experimental Section

Materials and Methods

Reagents were purchased from local suppliers and used without further purification. Anhydrous dichloromethane (DCM), anhydrous methanol (MeOH), anhydrous THF, hexane, and silica gel for column chromatography were purchased from Acros Organics. Ethyl acetate, DCM and MeOH reagent grade were attained from Fischer Scientific. All amino acids used are natural. Boc-β-Ala-OH, L-Phe-OMe.HCl, Boc-Gly-OH, and Boc-His(Bzl)-OH were purchased from Sigma–Aldrich. Triethylamine (TEA) and diisopropylethylamine (DIPEA) were obtained from Fluka, while trifluoroacetic acid (TFA) was purchased from Panreac. Thin Layer Chromatography (polyester, silica 60, UV 254) were obtained from Albet. NMR data were recorded with Bruker 500 MHz spectrometer and chemical shifts were reported as ppm. Mass spectroscopy spectra were obtained on Waters Quattro micro API LC/MS/MS in the positive mode at Michigan State University (USA).

Synthesis of Boc-His(Bn)-OMe (2)

Boc-His(Bn)-OH **1** (1 g; 2.9 mmol) was dissolved in anhydrous DCM (10 ml) and CDI (0.56 g, 3.5 mmol) was added. The reaction mixture was stirred at room temperature for 1 h after which anhydrous methanol (5 ml) was added. The reaction was stirred for 18 h after which the volatiles were evaporated on a rotavap. The residue was purified by column chromatography using DCM:MeOH (9:1) as an eluent. The

desired product was collected as colorless oil in 91% yield (0.95 g). The structure of **2** was confirmed by NMR (proton, carbon and COSY) and MS. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.43 (s, 9H), 2.97–3.06 (m, 2H), 3.49 (s, 1H), 3.64 (s, 3H), 4.45 (d, $J = 10.12$, 1H), 5.06 (s, 1H), 5.32 (s, 1H), 6.64 (s, 1H), 7.12 (d, $J = 5.12$, 2H), 7.31–7.36 (m, 3H), 7.45 (d, $J = 5.22$, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C 172.47, 155.57, 137.70, 137.08, 135.89, 128.97, 128.30, 127.23, 116.95, 79.55, 53.57, 52.11, 50.87, 30.16, 29.67, 28.31; MS (ESI). Calculated for $C_{19}H_{25}N_3O_4$: Theoretical mass: 359.18; Found: m/z $[M+H]^+$: 360.1.

Synthesis of Boc-Gly-His(Bn)-OMe (5)

Boc-His (Bn)-OMe **2** (0.6 g, 1.7 mmol) was dissolved in DCM (10 ml) and TFA (2 ml) was added. Three drops of triisopropylsilane (TIPS) was added as a radical scavenger. The reaction mixture was stirred at room temperature for 3 h until the disappearance of the starting material as indicated by TLC. The volatiles were evaporated on a rotavap. The resulting residue was watched with hexane (10 ml × 3) to remove residual TFA, and dried on high vacuum pump. The formation of the desired product [H-His(Bn)-OMe, **3**] was confirmed by was confirmed by MS: MS (ESI). Calculated for $C_{14}H_{17}N_3O_2$: Theoretical mass: 259.13; Found: m/z $[M+H]^+$: 260.1. The dried residue was dissolved in DCM (10 ml), and DIPEA (1 ml) was added. In a separate round bottom flask, Boc-Gly-OH **4** (0.35 g, 2.0 mmol) was dissolved in anhydrous DCM (20 ml), and CDI (0.4 g, 2.4 mmol) was added. The reaction mixture was stirred at room temperature for 1 h after which the H-His(Bn)-OMe **3** mixture was added. The mixture was stirred at room temperature for 18 h after which the volatiles were evaporated. The resulting residue was purified by column chromatography using DCM:MeOH (9:1) as an eluent. The desired product was collected as colorless oil in 86% (0.6 g). The structure was confirmed by NMR (proton, carbon, COSY) and MS. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.45 (s, 9H), 1.77 (s, 1H), 3.00–3.12 (m, 2H), 3.64 (s, 3H), 3.88 (d, $J = 5$, 2H), 4.80–4.83 (m, 1H), 5.05 (s, 2H), 5.20 (s, 1H), 6.67 (s, 1H), 7.12–7.14 (m, 2H), 7.31–7.39 (m, 3H), 7.44 (s, 1H), 7.62 (d, $J = 10$, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C 171.66, 169.12, 155.73, 137.82, 137.14, 135.97, 129.01, 128.34, 127.34, 127.22, 117.02, 79.95, 53.44, 52.37, 52.26,50.83, 43.99, 29.56,28.34. MS (ESI). Calculated for $C_{21}H_{28}N_4O_5$: Theoretical mass: 416.21; Found: m/z $[M+H]^+$: 417.1.

Synthesis of H-Gly-His(Bn)-OMe (6)

Boc-Gly-His(Bn)-OMe **5** (0.15 g, 0.36 mmol) was dissolved in DCM (5 ml) and TFA (1 ml) was added. TIPS was added as a radical scavenger. The reaction mixture was stirred at room temperature for 3 h until the disappearance of the starting material. The volatiles were evaporated on a rotavap and the residue was dried on a high vacuum pump. The dried residue was purified by column chromatography using DCM:MeOH (8:2) as an eluent. The desired product was collected in 87% yield (130 mg). The structure was confirmed by NMR (proton, carbon, COSY) and MS. ^{1}H-NMR (500 MHz, CD_3OD) δ_H 2.95–3.05 (m, 2H), 3.22 (s, 2H), 3.32 (s, 3H), 4.71 (t, $J = 15.21$, 1H), 5.16 (d, $J = 5.14$, 1H), 6.90 (s, 1H), 7.22–7.37 (m, H), 7.42 (s, 1H). ^{13}C-NMR (75 MHz, CD_3OD) δ_C 171.71, 137.16, 136.89, 128.53, 127.77, 127.19, 117.46, 52.74, 51.37, 50.21, 43.61, 41.84, 29.56. MS (ESI). Calculated for $C_{16}H_{20}N_4O_3$: Theoretical mass: 316.15; Found: m/z $[M+H]^+$: 317.2.

Synthesis of Boc-Gly-His-OMe (7)

Boc-Gly-His(Bn)-OMe **5** (0.12 g, 0.29 mmol) was dissolved in methanol (10 ml), and the mixture was cooled to 0 °C in an ice bath. Pd/C (12 mg) was added to the reaction mixture which was subjected to hydrogenation for 18 h. The reaction mixture was then filtered on a Celite bed, and methanol was evaporated. The resulting residue was purified by column chromatography using DCM:MeOH (9:1). The desired product was obtained in 96% (90 mg). The structure was confirmed by NMR (proton, carbon, COSY) and MS. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.46 (s, 9H), 3.11–3.25 (m, 2H), 3.0 (s, 1H), 3.72 (s, 3H), 3.82 (d, $J = 5.24$, 2H), 4.82 (dd, $J = 15.21$–3.02, 1H), 5.34 (s, 1H), 6.80 (s, 1H), 7.37 (s, 1H), 7.96 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C 171.45, 169.18, 156.66, 135.35, 80.56, 53.44, 52.57, 50.79, 44.38, 28.32. MS (ESI). Calculated for $C_{14}H_{22}N_4O_5$: Theoretical mass: 326.16; Found: m/z $[M+H]^+$: 327.1.

Synthesis of Boc-β-Ala-Phe-OMe (10)

Boc-Ala-OH **8** (1 g, 5.3 mmol) was dissolved in anhydrous dichloromethane (50 ml) and carbonyldiimidazole (CDI) (1 g, 6.34 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. In a separate reaction flask, L-Phe-OMe. HCl **9** (1.37 g, 6.34 mmol) is suspended in anhydrous dichloromethane (20 ml), and DIPEA (1.23 ml) was added. The solution was stirred at room temperature for 30 min, after which it is added to the Boc-Ala-OH mixture. The reaction mixture was stirred at room temperature for 6 h until TLC indicates the disappearance of starting material and appearance of product. DCM was evaporated and resulting residue was dissolved in ethyl acetate. The mixture was then washed with dilute aqueous HCl solution (0.1 M), saturated aqueous. sodium bicarbonate solution, and brine. The organic layer was collected, dried on anhydrous sodium sulfate, and volatiles are evaporated on RotaVap. The collected product was purified by column chromatography using EtOAc:Hexane (2:1). The desired product **10** was collected

in 94% yield (1.7 g) as colorless viscous oil. The structure of **10** was confirmed by NMR (proton, carbon, and COSY) and MS. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.43 (s, 9H), 2.37 (t, J = 10.32, 2H), 3.04–3.18 (m, 2H), 3.37 (dd, J = 15.25–5.15, 2H), 3.79 (s, 3H), 3.85–3.88 (m, 1H), 5.08 (s, 1H), 6.00 (d, J = 10.05, 1H), 7.08–7.10 (m, 2H), 7.23–7.34 (m, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C 171.90, 171.06, 156.02, 135.71, 129.17, 128.63, 127.27, 53.08, 52.41, 37.87, 36.49, 36.09, 28.39. MS (ESI). Calculated for $C_{18}H_{26}N_2O_5$: Theoretical mass: 350.18; Found: m/z $[M+Na]^+$: 373.1.

Synthesis of Boc-β-Ala-Phe-OH (11)

Dipeptide **10** (200 mg, 0.57 mmol) was dissolved in THF (10 ml) and the solution was cooled to 4 °C. An aqueous solution of 1 M LiOH (3 ml) was added dropwise, and the reaction mixture is stirred at 4 °C. The progress of the reaction was followed by TLC. The pH of solution was brought to 3–4 by the dropwise addition of 1 M aqueous HCl solution. THF was evaporated on a rotavap, the product was extracted by ethyl acetate. The ethyl acetate layer is collected and dried on anhydrous sodium sulfate. Ethyl acetate was evaporated, and product was purified by column chromatography using DCM:MeOH (9:1) as an eluent. The structure of the desired product **11** was confirmed by NMR (proton, carbon, and COSY) and MS. The product was collected in 92% yield (176 mg) as a colorless substance. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.44 (s, 9H), 2.39 (s, 2H), 3.04–3.07 (m, 1H), 3.20–3.36 (m, 4H), 4.83 (d, J = 5.42, 1H), 5.17 (s, 1H), 6.59 (d, J = 5.23, 1H), 7.16–7.29 (m, 4H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C 173.67, 171.38, 156.66, 136.06, 129.43, 128.50, 127.06, 79.93, 53.32, 37.46, 36.95, 33.61, 29.70, 28.39. MS (ESI). Calculated for $C_{17}H_{24}N_2O_5$: Theoretical mass: 336.17; Found: m/z $[M+Na]^+$: 359.1.

Synthesis of H- β-Ala-Phe-OMe (12)

Dipeptide **10** (200 mg, 0.57 mmol) was dissolved in DCM (10 ml) and trifluoroacetic acid (TFA) (1 ml) was added. Triisopropylsilane (TIPS) (50 µl) was added as a radical scavenger. The reaction mixture was stirred at room temperature for 2 h, or until the disappearance of reactants and appearance of the product. The volatiles were first evaporated on a rotavap, and then on a vacuum pump. The resulting residue was purified by column chromatography using DCM:MeOH (8:2) as an eluent. The desired colorless product was collected in 88% yield (174 mg). The structure of the desired product **12** was confirmed by NMR (proton, carbon, COSY) and MS. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 2.52–2.67 (m, 2H), 2.93–3.17 (m, 4H), 3.66 (s, 3H), 4.72–4.79 (m, 1H), 7.13–7.28 (m, 5H), 7.63 (d, J = 10.31, 1H), 8.08 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C 172.59, 170.73, 1662.16, 161.88, 136.01, 129.11, 128.56, 127.10, 117.48, 114.88, 53.88, 52.50, 37.40, 36.36, 31.87. MS (ESI). Calculated for $C_{13}H_{18}N_2O_3$: Theoretical mass: 250.13; Found: m/z $[M+H]^+$: 251.2.

Synthesis of Boc-β-Ala-His(Bn)-OMe (13)

Boc-His(Bn)-OMe **2** (0.7 g, 1.95 mmol) was dissolved in DCM (10 ml) and TFA (2 ml) was added. The reaction mixture was stirred at room temperature for 3 h until the disappearance of the starting material. The formation of the desired product **3** was confirmed by MS. The volatiles were evaporated on a rotavap and the residue was dried on a high vacuum pump. The dried residue was dissolved in DCM (10 ml) and DIPEA (1 ml) was added. In a separate round bottom flask, Boc-Ala-OH **8** (0.44 g, 2.34 mmol) was dissolved in anhydrous DCM (20 ml), and CDI (0.46 g, 2.80 mmol) was added. The reaction mixture was stirred at room temperature for 1 h after which the H-His(Bn)-OMe **3** mixture was added. The mixture was stirred at room temperature for 18 h after which the volatiles were evaporated. The resulting residue was purified by column chromatography using DCM:MeOH (9:1) as an eluent. The desired product was collected as colorless oil in 83% (0.7 g). The structure was confirmed by NMR (proton, carbon, COSY) and MS. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.42 (s, 9H), 1.65 (s, 1H), 2.43 (dd, J = 15.12–5.21, 2H), 3.00–3.07 (m, 2H), 4.43 (dd, J = 10.31–5.12, 2H), 3.63 (s, 3H), 4.80 (d, J = 5.25, 1H), 5.04 (s, 2H), 5.75 (s, 2H), 6.63 (s, 1H), 7.23 (d, J = 5.31, 1H), 7.32–7.37 (m, 3H), 7.46 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C 171.79, 137.45, 129.00, 128.35, 127.71, 116.93, 52.54, 52.24, 50.85, 36.4, 36.23, 29.39, 28.44. MS (ESI). Calculated for $C_{22}H_{30}N_4O_5$: Theoretical mass: 430.22; Found: m/z $[M+H]^+$: 431.2.

Synthesis of Boc-β-Ala-His-OMe (14)

Boc-Ala-His(Bn)-OMe **13** (0.15 g, 0.35 mmol) was dissolved in methanol (10 ml), and the mixture was cooled to 0 °C in an ice bath. Pd/C (15 mg) was added to the reaction mixture which was subjected to hydrogenation for 18 h. The reaction mixture was then filtered on a Celite bed, and methanol was evaporated. The resulting residue was purified by column chromatography using DCM:MeOH (9:1). The desired product **14** was obtained in 92% (110 mg). The structure was confirmed by NMR (proton, carbon, COSY) and MS. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.27 (s, 9H), 2.44 (t, J = 10.15, 2H), 3.12 (t, J = 5.42, 2H), 3.39–3.44 (m, 2H), 3.49 (s, 1H), 3.71 (s, 3H), 3.70–3.82 (m, 1H), 5.58 (s, 1H), 6.80 (s, 1H), 7.19 (d, J = 10.23, 1H), 7.56 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C 171.85, 171.58, 156.19, 135.22, 52.61, 52.44, 50.75, 36.92, 36.52, 28.93, 28.43. MS (ESI). Calculated for $C_{15}H_{24}N_4O_5$: Theoretical mass: 340.17; Found: m/z $[M+H]^+$: 341.1.

Synthesis of H-β-Ala-His(Bn)-OMe (15)

Boc-Ala-His(Bn)-OMe **13** (0.2 g, 0.46 mmol) was dissolved in DCM (5 ml) and TFA (1 ml) was added. TIPS (50 µl) was added as a radical scavenger. The reaction mixture was stirred at room temperature for 3 h till the disappearance of the starting material. The volatiles were evaporated on a rotavap and the residue was dried on a high vacuum pump. The dried residue was purified by column chromatography using DCM:MeOH (8:2) as an eluent. The desired product was collected in 85% yield (129 mg). The structure was confirmed by NMR (proton, carbon, COSY) and MS. ^{1}H-NMR (500 MHz, CD_3OD) δ_H 2.60 (dd, $J = 10.31–5.02$, 2H), 3.02–3.19 (m, 4H), 3.31–3.32 (m, 1H), 4.70–4.72 (m, 1H), 5.31 (s, 2H), 7.18 (s, 1H), 7.32–742 (m, H), 8.82 (s, 1H). ^{13}C-NMR (75 MHz, CD_3OD) δ_C 171.19, 170.69, 135.85, 135.41, 128.77, 128.35, 127.57, 118.72, 52.06, 51.57, 51.40, 35.41, 31.23, 27.86. MS (ESI). Calculated for $C_{17}H_{22}N_4O_3$: Theoretical mass: 330.17; Found: *m*/*z* $[M+H]^+$: 331.1.

Synthesis of Boc-Gly-Phe-OMe (16)

Boc-Gly-OH **4** (1 g, 5.7 mmol) was dissolved in anhydrous dichloromethane (30 ml) and carbonyl diimidazole (CDI) (1.11 g, 6.85 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. In a separate reaction flask, L-Phe-OMe. HCl **9** (1.47 g, 6.85 mmol) is suspended in anhydrous dichloromethane (20 ml), and DIPEA (1.4 ml) was added. The solution was stirred at room temperature for 30 min, after which it is added to the Boc-Gly-OH mixture. The reaction mixture was stirred at room temperature for 6 h until TLC indicates the disappearance of starting material and appearance of product. DCM was evaporated and resulting residue was dissolved in ethyl acetate. The mixture was then washed with dilute aqueous HCl solution (0.1 M), saturated aqueous sodium bicarbonate solution, and brine. The organic layer was collected, dried on anhydrous sodium sulfate, and volatiles are evaporated on RotaVap. The collected product was purified by column chromatography using EtOAc:Hexane (2:1). The desired product **16** was collected in 93% yield (1.8 g) as colorless viscous oil. The structure of the (3) was confirmed by NMR (proton, carbon, and COSY) and MS. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.45 (s, 9H), 3.07–3.16 (m, 2H), 3.72 (s, 3H), 4.87–4.91 (m, 1H), 5.10 (s, 1H), 6.53 (d, $J = 5.21$, 1H), 7.1–7.15 (m, 2H), 7.2–7.31 (m, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C 171.66, 169.03, 135.58, 129.23, 128.65, 127.19, 53.04, 52.38,44.21, 37.88, 28.27. MS (ESI). Calculated for $C_{17}H_{24}N_2O_5$: Theoretical mass: 336.17; Found: *m*/*z* $[M+H]^+$: 337.12.

Synthesis of Boc-Gly-Phe-OH (17)

Boc-Gly-Phe-OMe **16** (200 mg, 0.6 mmol) was dissolved in THF (10 ml) and the solution was cooled to 4 °C. An aqueous solution of 1 M LiOH (3 ml) was added dropwise, and the reaction mixture is stirred at 4 °C. The progress of the reaction was followed by TLC. The pH of solution was brought to 3–4 by the dropwise addition of 1 M aqueous HCl solution. THF was evaporated on a rotavap, and the product was extracted by ethyl acetate. The ethyl acetate layer was collected and dried on anhydrous sodium sulfate. Ethyl acetate was evaporated, and the product was purified by column chromatography using DCM: MeOH (9:1) as an eluent. The product was collected in 97% (190 mg) as a colorless substance. The structure of the desired product **17** was confirmed by NMR (proton, carbon, and COSY). ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.43 (s, 9H), 3.01–3.21 (m, 2H), 3.61–3.91 (m, 3H), 4.82 (s, 1H), 4.90 (s, 1H), 5.74 (s, 1H), 6.03 (s, 1H), 6.88 (s, 1H), 7.15–7.27 (m, H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C 173.98, 169.75, 156.35, 135.81, 129.37, 128.56, 127.07, 80.58, 53.21, 43.9, 37.39, 28.3.

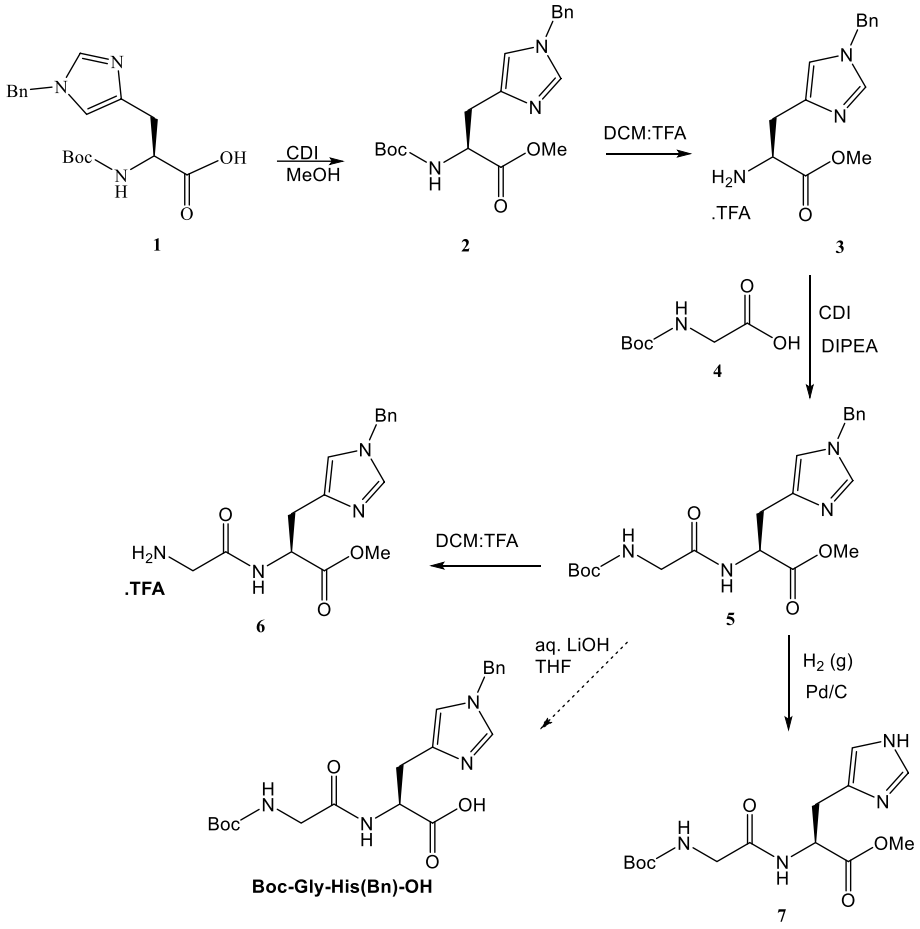
Synthesis of H-Gly-Phe-OMe (18)

Dipeptide **16** (200 mg, 0.6 mmol) was dissolved in DCM (10 ml) and trifluoroacetic acid (TFA) (1 ml) was added. Triisopropylsilane (TIPS) (50 µl) was added as a radical scavenger. The reaction mixture was stirred at room temperature for 2 h, or till the disappearance of reactants and appearance of the product. The volatiles were first evaporated on a rotavap, and then on a vacuum pump. The resulting residue was purified by column chromatography using DCM:MeOH (8:2) as an eluent. The structure of the desired product **18** was confirmed by NMR (proton, carbon, COSY) and MS. The desired colorless product was collected in 90% yield (173 mg). ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 2.93–3.08 (m, 2H), 3.57 (s, 3H), 3.63 (d, $J = 15.21$, 1H), 3.79 (d, $J = 15.12$, 1H), 4.76 (dd, $J = 10.32–5.01$, 1H), 7.09–7.27 (m, 6H), 7.93 (d, $J = 10.21$, 1H), 8.10 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C 172.12, 166.30, 162.24, 161.99, 135.86, 129.14, 128.52, 127.05, 54.03, 54.07, 52.41, 40.68, 37.47. MS (ESI). Calculated for $C_{12}H_{16}N_2O_3$: Theoretical mass: 236.17; Found: *m*/*z* $[M+H]^+$: 237.2.

Results

Synthesis of Gly-His Series

The first synthesized series of CAR analogues was based on the Glycine-Histidine (Gly-His) dipeptide. The overall synthetic scheme is depicted in Scheme 1. The rationale behind establishing this homologous series was to examine



Scheme 1 Overall synthetic route for the synthesis of Gly-His analogues

the significance of β-Ala on CAR's biological activity. A glycine moiety with one methylene (CH_2) group shorter substituted β-Ala.

Synthesis of the Gly-His series started by protecting the carboxyl group of Boc-His(Bn)-OH (**1**) in the form of methyl ester (Scheme 1). Compound **1** was coupled with methanol using carbonyl diimidazole (CDI) as a coupling reagent to produce the fully protected His analogue **3**. The success of the synthesis was confirmed by NMR (^{1}H, ^{13}C, and ^{1}H–^{1}H COSY) and mass spectroscopy. The ^{1}H-NMR spectrum confirmed the appearance of a singlet at 3.64 ppm integrated for three protons corresponding to the methyl ester (OCH_3) group. In addition, the ^{1}H-NMR spectrum showed a singlet at 1.43 ppm integrated for the nine protons of the *tert*-butyl group of Boc, a multiplet at 2.97–3.06 ppm integrated for two protons of the CH_2 group, and a multiplet at 4.51–4.57 ppm integrated for one proton of the CH group. ^{13}C-NMR further confirmed the success of esterification. The connectivity of the atoms was assessed by collecting ^{1}H–^{1}H COSY spectrum and correlating the various peaks. Additional supportive data was obtained from the mass spectroscopy spectrum that showed at peak at m/z 360 which corresponds to $(M+H)^+$.

The amino group of compound **2** was selectively deprotected upon treatment with trifluoroacetic acid (TFA) in DCM for 3 h at room temperature to produce His analogue **3** (Scheme 1). Triisopropylsilane (TIPS) was used as a radical scavenger. The residue obtained following the evaporation of the volatiles was washed with hexane to afford the Boc-free His **3** in 96% yield. TLC analysis showed a smaller R_f for **3** than **2**. This increase in polarity was attributed to the regeneration of the amino-terminal indicating a successful reaction. The success of the reaction was further confirmed by the mass spectroscopy where a $(M+H)^+$ peak appeared at 260 matching the molecular mass of **3**.

The fully protected dipeptide Boc-Gly-His(Bn)-OMe **5** was next synthesized by coupling His **3** and the Boc-protected glycine (Boc-Gly-OH) **4** using CDI as a coupling reagent (Scheme 1). NMR and mass spectroscopic data confirmed the successful conjugation. The ^{1}H-NMR spectrum showed a singlet at 1.45 ppm integrated for nine protons corresponding to *tert*-butyl group of Boc, two multiplets at 2.99–3.12 and 4.80–4.85 ppm corresponding to the CH_2 and CH groups of His, respectively, a singlet at 5.05 ppm corresponding to the CH_2 group of the benzyl group, and a doublet at 3.88 ppm corresponding to the CH_2 group of Gly.

^{13}C-NMR showed three peaks at 169, 171, and 173 ppm corresponding to the three C=O groups in the molecule. Further confirmation for the success of the reaction was obtained from mass spectroscopy which depicted a m/z peak at 417 corresponding to $(M+H)^+$.

Analogue **6**, with a deprotected amino group, was afforded upon the treatment of **5** with TFA/DCM in the presence of triisopropylsilane (TIPS) as a radical scavenger. The disappearance of the peak at 1.4 ppm in the ^{1}H-NMR spectrum and the peaks at 28 and 52.3 ppm in the ^{13}C-NMR spectrum suggested the selective removal of the Boc group. Mass spectroscopy showed a peak at m/z 317 corresponding to $(M+H)^+$ of **6**, thus further supporting the successful formation of **6** (Scheme 1).

The orthogonal deprotection of the imidazole ring of His was achieved by subjecting compound **5** to catalytic hydrogenation conditions using 10% Pd/C as a catalyst (Scheme 1) (Li et al. 2016). The progress of the reaction was traced by TLC, and the reaction was stopped when all starting material disappeared. The desired product **7** was obtained in 96% yield following purification by column chromatography. NMR spectral data confirmed the success of the chemical transformation. In the ^{1}H-NMR, the peaks that integrate for the five aromatic protons in the 7–7.5 ppm region and the singlet at 5.05 ppm that integrate for the two methylene protons of the benzyl group disappeared. This was correlated with data from of the ^{13}C-NMR spectrum that showed the disappearance of the peaks between 120 and 150 ppm corresponding to the carbons of the phenyl group, as well as the peak at 29.5 ppm that corresponds to the methylene group carbon. Further support for the success of the reaction came from mass spectroscopy that presented a peak at m/z 327 corresponding to $(M+H)^+$.

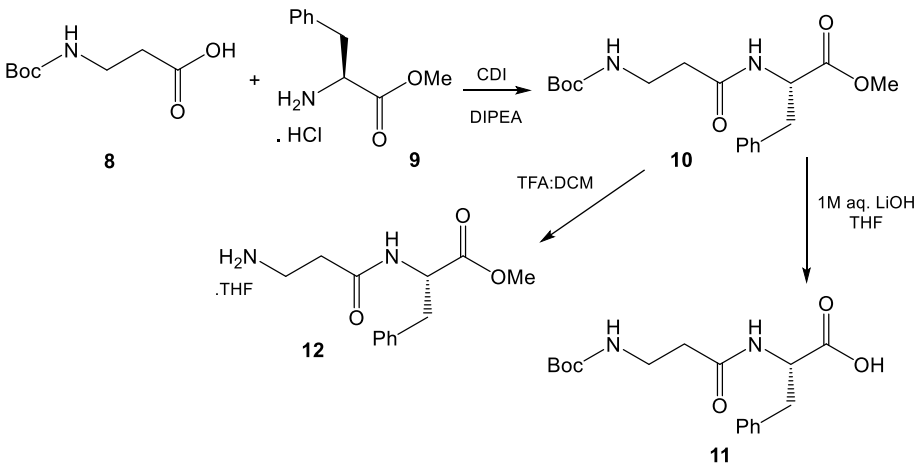
Boc-Gly-His(Bn)-OH was approached through the hydrolysis of the methyl ester functionality of dipeptide **5** under basic conditions. While the chemical transformation was successful as indicated by mass spectroscopy, an analytical sample was not obtained and therefore NMR spectra were not clean.

Synthesis of β-Ala-Phe Series

The next synthesized series of carnosine analogues was based on β-Ala-Phe (Scheme 2). The rationale behind synthesizing this series was to investigate the role of the imidazole ring in CAR's biological activity. Therefore, His was substituted for Phe with a phenyl ring in the side chain instead of the imidazole one.

The fully protected dipeptide **10** was synthesized as depicted in Scheme 2. Following the activation of carboxyl group of Boc-β-Ala-OH **8** using CDI, H-Phe-OMe **9** was added leading to the generation of the desired product **10**. The structure of **10** was confirmed by NMR and mass spectroscopy. In the ^{1}H-NMR, the peaks at 1.43, 2.73, and 3.36 ppm corresponded to the Boc and the two CH_2 groups of β-Ala, respectively, while the peaks at 3.15, 3.73, and 4.87 ppm corresponded to CH_2, OCH_3, and CH groups of Phe, respectively. In addition, the multiplets at 7.08–7.31 ppm integrated for the five aromatic protons of the phenyl group. The ^{1}H–^{1}H COSY spectrum assisted in assigning the peaks to the respective protons. Furthermore, the ^{13}C-NMR provided the right number and chemical shifts of the carbon atoms with carbonyl groups of the amide and ester functionalities appearing at 171.06 and 171.9, respectively. Finally, the mass spectroscopy spectrum collected for **10** depicted a peak at m/z 373 corresponding to $(M+23)^+$.

Dipeptide **11** with a free carboxyl group was obtained by base hydrolysis of the methyl ester group using an aqueous lithium hydroxide solution in THF at 0 °C (Scheme 2). The successful generation of compound **11** was supported by the disappearance of the peak at 3.73 pm in the ^{1}H-NMR spectrum and the peak at 52.4 ppm in the ^{13}C-NMR spectrum corresponding to CH_3 of the methyl ester functionality.



Scheme 2 Overall synthetic scheme for the preparation of β-Ala-Phe series

Furthermore, mass spectroscopy showed a peak at m/z 359 corresponding to the $(M+23)^+$.

Finally, analogue **12** with a free amino group was obtained by reacting dipeptide **10** with TFA in the presence of triisopropylsilane (TIPS) as a radical scavenger (Scheme 2). The product was purified by column chromatography using DCM: MeOH (8:2) as an eluent. The disappearance of the peak at 1.43 ppm in the ^{1}H-NMR spectrum and the peak at 28.39 ppm in the ^{13}C-NMR spectrum provided evidence for the success of the hydrolysis. In addition, the mass spectrum collected for compound **12** showed a peak at 3/z 251 corresponding to $(M+H)^+$.

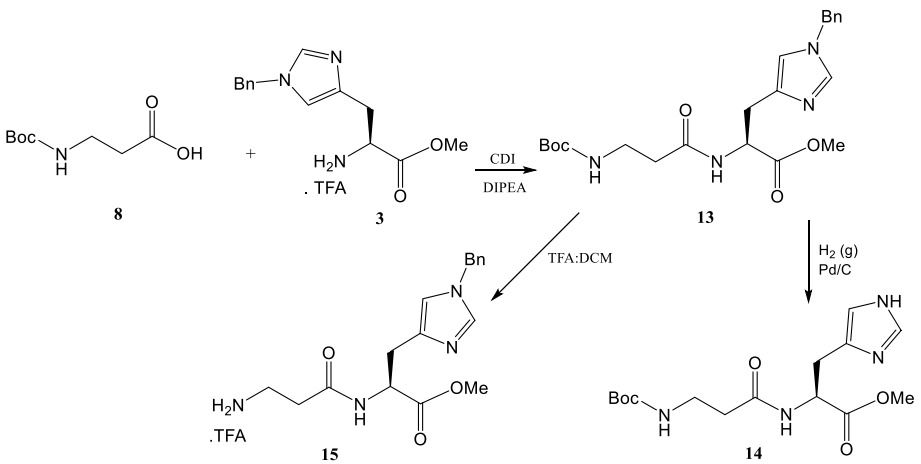
Synthesis of the β-Ala-His Series

In an attempt to investigate the pharmacophoric functional groups of carnosine, a series of β-Ala-His analogues was synthesized with orthogonally exposed functional groups. The overall synthetic routes to access these analogues is depicted in Scheme 3.

Boc-β-Ala-His(Bn)-OMe **13**, the fully protected carnosine analogue, was synthesized by coupling Boc-β-Ala **8** with H-His(Bn)-OMe **3** using CDI as a coupling agent. Dipeptide **13** was produced in 83% yield following purification by column chromatography using EtOAc:Hexane (9:1) as an eluent. All collected spectroscopic data supported the production of **13**. In the ^{1}H-NMR spectrum, the peaks at 2.44 and 3.42 ppm which integrated for two protons each correspond to the two methylene groups of Ala, while the peaks at 3.03 and 4.79 ppm corresponds to the CH_2 and CH groups of His. In addition, the singlet at 1.42 ppm which integrated for nine protons, the singlet at 3.43 ppm which integrated for three protons, and the peaks in the 7–7.4 ppm region which integrated for five protons corresponded to the $–C(CH_3)_3$, $–OCH_3$, and the phenyl group, respectively. The singlet at 5.04 ppm corresponded to the methylene of the benzyl protecting group. The ^{13}C-NMR also supported the generation of compound **13**. The connectivity of the atoms was deduced from the analysis of the ^{1}H–^{1}H COSY spectrum. Additional confirmative indication for the formation of the fully protected analogue came from the mass spectroscopy spectrum that showed a peak at m/z 431 corresponding to the $(M+H)^+$.

Orthogonal deprotection procedures started by subjecting dipeptide **13** to catalytic hydrogenation using Pd/C as a catalyst which led to the selective removal of the benzyl protecting group from the imidazole ring (Scheme 3). The crude product was purified by column chromatography to afford the desired dipeptide **14** with an unprotected imidazole ring in 92% yield. The disappearance of the singlet at 5.02 ppm and the peaks in the aromatic region which correspond to the methylene and phenyl groups of benzyl, respectively, confirmed the successful deprotection. In ^{13}C-NMR, the disappearance of the four peaks in the 116–130 ppm and the peak at 79 ppm corresponding to the carbon atoms of the phenyl and the methylene groups of benzyl, respectively, added evidence to the success of the reaction. The persistence of the peaks at 1.44 and 3.7 ppm corresponding to the *tert*-butyl group of Boc and the methyl group of the methyl ester, respectively, reinforced the orthogonality of the applied deprotection approach. Additional evidence was collected from the mass spectroscopy which presented a peak at m/z 341 corresponding to $(M+H)^+$.

Orthogonal deprotection procedures continued by the selective removal of the Boc group protecting the N-terminal of dipeptide **13**. This was accomplished by treating compound **13** with TFA, and analogue **15** with a free amino group was generated in 85% yield after purification on a silica column using DCM:MeOH (8:2) as an eleuent (Scheme 3). Analysis of the NMR spectrum showed the disappearance of the peak at 1.42 ppm in the ^{1}H-NMR spectrum and the peak at 28.44 ppm in the ^{13}C-NMR spectrum provided evidence for the removal of Boc group. In addition, the existence of a peak at 5.29 ppm and another at 3.66 ppm



Scheme 3 Overall synthesis scheme for the preparation of β-Ala-His series

which are assigned to the CH_2 of the benzyl group and the CH_3 of the methyl ester functionality, respectively, proved the orthogonality of the deprotection reaction. The m/z peak at 331 of the mass spectroscopy spectrum of compound **15** which corresponds to $(M+H)^+$ further validated the proposed chemical structure for **15**.

Synthesis of the Gly-Phe Series

The Glycine-Phenylalanine (Gly-Phe) series was the final set of synthesized carnosine analogues that can be used as a "negative control" when assessing CAR's biological activity. The overall synthetic scheme is shown in Scheme 4.

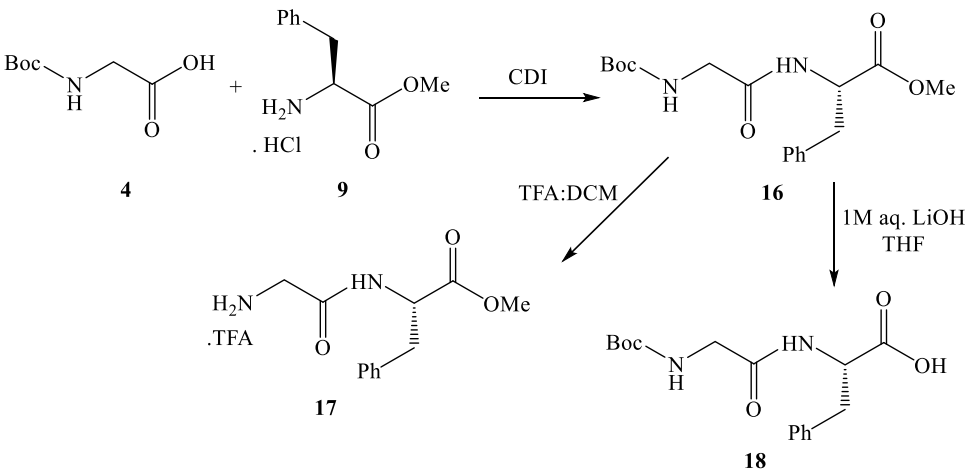
Boc-Gly-Phe-OMe **16** was prepared by reacting mixture of Boc-Gly-OH **4** and H-Phe-OMe **9** in anhydrous dichloromethane in the presence of CDI as a coupling reagent (Scheme 4.18). The formation of **16** was tracked by TLC. The product was purified by column chromatography using EtOAc:Hexane (2:1) following the disappearance of starting material and appearance of product. The desired product was obtained in 93% yield demonstrating the efficacy of the applied synthetic approach. The OCH_3, CH_2, CH, and Ph groups from Phe produced peaks at 1.45, 3.14, 4.88, and 7.0–7.3 ppm, respectively, in the ^{1}H-NMR spectrum. In addition, the *tert*-butyl group of Boc and the methylene group of Gly showed peaks at 1.45 and 3.75 ppm, respectively. Peaks in the ^{13}C-NMR corroborated the production of **16**. The peaks at 169 and 171 ppm were attributed to the carbonyl groups of the amide and the ester functionality, respectively, while the four peaks between 127 and 135 ppm were assigned to the phenyl group carbon atoms. On the other hand, the peaks at 28, 37, 44, 52 and 53 ppm were attributed to the $-C(CH_3)_3$, CH_2 (Phe), CH (Phe), CH_2 (Gly), and OCH_3 groups respectively. Mass spectroscopy further confirmed the structure of **16** showing a peak at m/z 337 corresponding to $(M+H)^+$.

Dipeptide Boc-Gly-Phe-OH **17** with a free carboxyl group at the C-terminal was synthesized by treating compound **16** with aqueous LiOH solution in THF (Scheme 4). The consumption of reactant **16** and the formation of a more polar compound as shown by TLC suggested a successful chemical transformation. Purification of the crude mixture by column chromatography yielded the desired product **17** in 97% yield. The disappearance of the peak at 3.72 ppm in the ^{1}H-NMR spectrum and the peak at 52 ppm of the ^{13}C-NMR spectrum, both of which are attributed to the methyl protecting group, validated the success of the reaction.

The final analogue to be synthesized was H-Gly-Phe-OMe with a free amino group at the N-terminal of the dipeptide. This required the selective removal of the of the Boc group under acidic group. The anticipated product **18** was obtained in 90% yield. Analysis of an analytical sample by NMR and mass spectroscopy confirmed the success of the reaction. The singlet at 1.45 ppm in the ^{1}H-NMR spectrum and the peak at 28 ppm in the ^{13}C-NMR spectrum, which correspond to the $-C(CH_3)_3$ group, disappeared. In addition, analysis of the product by mass spectroscopy validated the structure of **18** by showing a peak at m/z 237 which matched $(M+H)^+$.

Discussion

Carnosine is an endogenous dipeptide widely and abundantly distributed in the muscle and nervous tissues of several animal species. Many functions have been proposed for this compound because of its antioxidant and metal ion-chelator properties. However, the therapeutic uses of the dipeptide are strongly limited by the mechanism governing its homeostasis. This fact has been the main reason for the synthesis of carnosine derivatives with interesting potentiality, but until now, there have been very few applications. The main goals reached by the carnosine functionalization



Scheme 4 Overall synthetic scheme for the preparation of the Gly-Phe series

have been avoiding or, at least, reducing the carnosinase hydrolysis, conferring a lipophilic character with the aim to aid the BBB-crossing, enhancing or, at least, maintaining the beneficial effects of the dipeptide, counterbalancing the side effects of the grafted compound, and aiming at the targeted delivery (Bellia et al. 2012).

In an attempt to elucidate the chemical constituents that are responsible for carnosine's anti-crosslinking activity, Hobart et al. tested the individual amino acids in carnosine (β-alanine and L-histidine) as well as modified forms of histidine (α-acetyl-histidine, L-methyl-histidine) and methylated carnosine (anserine) (Hobart et al. 2004). β-Alanine showed anti-crosslinking activity but less than that of carnosine, suggesting that the α-amino group is required in preventing protein crosslinking. Interestingly, histidine, which has both α-amino and imidazolium groups, was more effective than carnosine. Acetylation of histidine's α-amino group or methylation of its imidazolium group abolished anti-crosslinking activity. The authors postulated that the primary amine was crucial as evidenced by the observed anti-crosslinking activity of β-alanine and the abolished activity of acetylated histidine with a blocked α-amine. The authors also suggested that imidazole play a supportive role in preventing glycation-induced crosslinking. Methylation at the N-1 position of imidazole of carnosine also diminished its anti-crosslinking activity of carnosine (Hobart et al. 2004). Despite these interesting findings, the study reached its conclusion based on assessing the anti-crossing activity of individual amino acids rather than carnosine analogues, which discards any potential synergistic effect resulting from linking β-alanine and histidine. Furthermore, histidine was found to be more effective than carnosine suggesting that β-alanine might be acting as an auxophore rather than part of the pharmacophore. Therefore, preparing a library of carnosine analogues such as the one prepared in this study would lead to a better understanding of the functional groups that are responsible for carnosine's anti-crosslinking activity.

It has been reported that the carboxylic group is important in the recognition of carnosine by the carnosinase enzymes (Unno et al. 2008). Thus, the conversion of carboxyl group into a different functionality such as an amide or ester might afford derivatives that are very stable to the carnosinase action, yet retaining some important biological functions. Several studies reported the synthesis of L-carnosine derivatives modified at the carboxyl group (Lanza et al. 2011; Bertinaria et al. 2011; Bellia et al. 2008). Bertinaria et al. reported the synthesis, physicochemical characterization, and biological activities of carnosine derivatives modified at the carboxyl end by an amide. These derivatives proved to be stable in human serum and thus can be useful as potential neuroprotective agents. In particular, the propyl amide derivative of carnosine was able to protect primary hippocampus neurons against HNE-induced death, showing a very significant increase in comparison to L-carnosine. In addition, the new derivative was able to cross the blood brain barrier (BBB) and to concentrate in the rat brain after intravenous administration. Besides the amide derivatives, an ethyl ester analogue of carnosine has been reported. Unlike the amide derivatives of carnosine which formed dimeric complexes with Cu(II), though with lower thermodynamic stability compared to L-carnosine, the ethyl ester analogue only formed monomeric species probably due to the lack of carboxylate (Lanza et al. 2011; Bertinaria et al. 2011). The series of CAR analogues prepared in the current study will offer an opportunity to re-visit the this topic and investigate the role that each functional group of L-carnosine plays in complexing to metals ion such as copper and zinc ions.

Castelletto et al. studied the effect of attachment of a bulky hydrophobic aromatic unit, namely *N*-(fluorenyl-9-methoxycarbonyl) (Fmoc), on the self-assembly of Fmoc-L-carnosine and its zinc-binding properties (Castelletto et al. 2011). The authors showed that Fmoc-L-carnosine formed well-defined amyloid fibrils containing β sheets above a critical aggregation concentration, and concluded that modifying the dipeptide by incorporation of a terminal Fmoc unit may improve the efficiency of carnosine in biotechnology applications, since Fmoc-L-carnosine can form fibers while still chelating metal ions. A similar study has been published recently assessing the ability of L-carnosine-derived Fmoc-tripeptides to form pH-sensitive and proteolytically stable supramolecular hydrogels (Mahapatra et al. 2017). Therefore, it would be interesting to investigate the effect of attaching a smaller, aliphatic, hydrophobic group like Boc on the self-assembly of carnosine fibers and its metal ion complexing capabilities. This feature is encountered in many of the carnosine analogues prepared in this study. Furthermore, the effect of varying the β-Ala chain length (i.e. the Gly series), hindering the carboxyl group (i.e. the methyl ester series) and masking the imidazole ring (i.e. the protected His and Phe series) would provide a deeper understanding of the factors affecting self-assembly of carnosine fibers and its hydrogelation in the presence of metal ions.

The broad spectrum biological activity of carnosine continues to entice scientist to develop novel analogues with improved pharmacokinetic profile. For example, Iacobini et al. recently studied the effect of carnosinol, a carnosinase-resistant and bioavailable carnosine derivative, on the onset and progression of diabetic nephropathy in *db*/*db* mice (Iacobini et al. 2017). Carnosinol was found to be selective and reactive as RCS sequestering agent. In addition, it is characterized by lack of toxicity and a favorable pharmacokinetic profile, related to recognition by human peptide transporter 1 as well as resistance to carnosinase. The molecule was effective in preventing the onset and halting progression of diabetic nephropathy by quenching RCS, thereby reducing the accumulation of their protein adducts and the consequent

inflammatory response. Therefore, a simple reduction of the carboxyl group of L-carnosine into a primary alcohol resulted in a novel compound that may represent a promising advanced glycation endproducts (AGE)-reducing approach for diabetic nephropathy therapy.

Conclusion

It is clear that research addressing novel derivatives of carnosine is far from over and plenty of questions are yet to be answered. The study presented in this study offers a feasible synthetic approach for the preparation of new analogues with orthogonally protected functional groups that might aid in addressing or answering many of the mysteries associated with the biological benefits of carnosine.

Acknowledgements We are grateful for Dr. Kamal Bouhadir from the American University of Beirut for helping in NMR experiments.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and Animal Rights The article does not contain any studies with human or animal subjects performed by any of the authors.

Informed Consent The authors declare that there is no informed consent in the article.

References

Aldini G, Carini M, Beretta G, Bradamante S, Maffei Facino R (2002) Carnosine is a quencher of 4-hydroxy-nonenal: through what mechanism of reaction? Biochem. Biophys Res Commun 298:699–706

Bauer K (2005) Carnosine and homocarnosine, the forgotten, enigmatic peptides of the brain. Neurochemical Res 30:1339–1345

Bellia F, Amorini AM, La Mendola D, Vecchio G, Tavazzi B, Giardina B et al (2008) New glycosidic derivatives of histidine-containing dipeptides with antioxidant properties and resistant to carnosinase activity. Eur J Med Chem 43:373–380

Bellia F, Vecchio G, Rizzarelli E (2012) Carnosine derivatives: new multifunctional drug-like molecules. Amino Acids 43:153–163

Bellia F, Oliveri V, Rizzarelli E, Vecchio G (2013) New derivative of carnosine for nanoparticle assemblies. Eur J Med Chem 70:225–232

Bertinaria M, Rolando B, Giorgis A, Montanaro G, Gasco A, Daniel PG et al (2011) Synthesis, physicochemical characterization, and biological activities of new carnosine derivatives stable in human serum as potential neuroprotective agents. J Med Chem 54:611–621

Bertinaria M, Rolando B, Giorgis M, Montanaro G, Marini E, Collino M et al (2012) Carnosine analogues containing NO-donor substructures: synthesis, physico-chemical characterization and preliminary pharmacological profile. Eur J Med Chem 54:103–112

Boldyrev AA (2000) Problems and perspectives in studying the biological role of carnosine. Biochemistry 65:751–756

Boldyrev AA, Gallant SC, Sukhich GT (1999) Carnosine: the protective, anti-aging peptide. Mol Asp Med 19:581–587

Castelletto V, Cheng G, Greenland BW, Hamley IW, Harris PJ (2011) Tuning the self-assembly of the bioactive dipeptide L-carnosine by incorporation of a bulky aromatic substituent. Langmuir 27:2980–2988

Craik DJ, Fairlie DP, Liras S, Price D (2013) The future of peptide-based drugs. Chem Biol Drug Design 81:136–147

Durmus Z, Kavas H, Baykal A, Sozeri H (2011) Synthesis and characterization of L-carnosine coated iron oxide nanoparticles. J Alloys Compd 509:2555–2561

Fosgerau K, Hoffmann T (2015) Peptide therapeutics: current status and future directions. Drug Discov Today 20:122–128

Guiotto A, Calderan A, Ruzza P, Borin G (2005) Carnosine and carnosine-related antioxidants: a review. Curr Med Chem 12:2293–2315

Hamley IW (2007) Peptide fibrillization. Angew Chem Int Ed 46:8128–8147

Hipkiss AR (2009) Carnosine and its possible roles in nutrition and health. Adv. Food Nutr Res 57:87–154

Hipkiss RA, Cartwright PC, Bromley C, Gross SR, Bill RM (2013) Carnosine: can understanding its actions on energy metabolism and protein homeostasis inform its therapeutic potential. Chem Cent J 7:38–46

Hobart LJ, Seibel I, Yeargans GS, Seidler NW (2004) Anti-crosslinking properties of carnosine: significance of histidine. Life Sci 75:1379–1389

Iacobini C, Menini S, Fantauzzi CB, Pesce CM, Giaccari A, Salomone E et al (2017) FL-926-16, a novel bioavailable carnosinase-resistant carnosine derivative, prevents onset and stops progression of diabetic nephropathy in db/db mice. Br J Pharmacol. https://doi.org/10.1111/bph.14070

Krpetić Z, Guerrini L, Larmour IA, Reglinski J, Faulds K, Graham D (2012) Importance of nanoparticle size in colorimetric and SERS-based multimodal trace detection of Ni(II) ions with functional gold nanoparticles. Small 8:707–714

Lanza V, Bellia F, D'Agata R, Grasso G, Rizzarelli E, Vecchio G (2011) New glycoside derivatives of carnosine and analogs resistant to carnosinase hydrolysis: synthesis and characterization of their copper(II) complexes. J Inorg Biochem 105:181–188

Li H, Chen J, Huang H, Feng J-J, Wang A-J, Shao L-X (2016) Green and facile synthesis of L-carnosine protected fluorescent gold nanoclusters for cellular imaging. Sens Actuators B 223:40–44

Mahapatra RD, Dey J, Weiss RG (2017) L-Carnosine-derived Fmoc-tripeptides forming pH-sensitive and proteolytically stable supramolecular hydrogels. Langmuir 33:12989–12999

Malkar VV, Mukherjee T, Kapoor S (2015) Carnosine induced formation of silver nanochains: a radiolytic study. Radiat Phys Chem 107:54–58

Orioli M, Vistoli G, Regazzoni L, Pedretti A, Lapolla A, Rossoni G et al (2011) Design, synthesis, ADME properties, and pharmacological activities of beta-alanyl-D-histidine (D-carnosine) prodrugs with improved bioavailability. ChemMedChem 6:1269–1282

Pegova A, Abe H, Boldyrev A (2000) Hydrolysis of carnosine and related compounds by mammalian carnosinases. Comp Biochem Physiol B 127:443–446

Saada MC, Montero JL, Vullo D, Scozzafava A, Winum JY, Supuran CT (2011) Carbonic anhydrase activators: gold nanoparticles coated with derivatized histamine, histidine, and carnosine show enhanced activatory effects on several mammalian isoforms. J Med Chem 54:1170–1177

Sachdeva S (2017) Peptides as 'Drugs': the journey so far. Int J Pept Res Ther 23:49–60

Teufel M, Saudek V, Ledig JP, Bernhardt A, Boularand S et al (2003) Sequence identification and characterization of human

carnosinase and a closely related non-specific dipeptidase. J Biol Chem 278:6521–6531

Unno H, Yamashita T, Ujita S, Okumura N, Otani H, Okumura A et al (2008) Structural basis for substrate recognition and hydrolysis by mouse carnosinase CN2. J Biol Chem 283:27289–27299

Vistoli G, Orioli M, Pedretti A, Regazzoni L, Canevotti R, Negrisoli G at al (2009) Design, synthesis, and evaluation of carnosine derivatives as selective and efficient sequestering agents of cytotoxic reactive carbonyl species. ChemMedChem 4:967–975

Vistoli G, Colzani M, Mazzolari A, Maddis DD, Grazioso G, Pedretti A, Carini M, Aldini G (2016) Future Med Chem 8:1721–1737