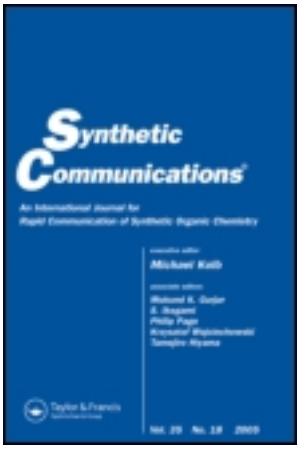

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New Nitrilotriacetic Acid and Ethylenediaminetetraacetic Acid Nitro Derivatives for the Synthesis of Bifunctional and Trifunctional Chelating Agents

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New Nitrilotriacetic Acid and Ethylenediaminetetraacetic Acid Nitro Derivatives for the Synthesis of Bifunctional and Trifunctional Chelating Agents

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Abstract: Dehydration of serine derivatives into didehydroamino acids and subsequent conjugate addition of nitromethane led to novel nitro derivatives of nitrilotriacetic acid and ethylenediaminetetraacetic acid. Straightforward transformations of these compounds allowed the synthesis of bifunctional and trifunctional metal-chelating agents via an Henry reaction.

Keywords: Chelates, Henry reaction, lanthanides, ligands, polyaminocarboxylates

INTRODUCTION

Polyaminocarboxylate chelates have the ability to form stable metal complexes with many metal ions such as nickel, copper, indium, technetium, and iron, in different oxidation states. Therefore, the development of bifunctional chelating agents that can be attached to macro(bio)molecules offers useful tools such as (1) probes of proteins structure,[1] (2) probes for molecular recognition,[2] (3) contrast agents for magnetic resonance,[3] (4) in vivo tumor imaging reagents,[4] and (5) radioimmunotherapy agents.[5] The purpose of designing trifunctional chelating agents is to combine these properties to achieve more complex and targeted applications.

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Biotinylated-EDTA–based trifunctional agents have been used for the preparation of radiolabeled antibodies that can be easily removed from blood with the aim to decrease side effects of radiotherapies.[6] As a second example, functionalization of phospholipids using a biotinylated-NTA–based trifunctional agent allows the organization of streptavidin and polyhistidine-tagged proteins around tubular structures offering valuable tools to structural biology.[7]

Although nitrilotriacetic acid (NTA)–based bifunctional chelating agents are usually synthesized from protected L-Lysine by alkylation with bromoacetic acid,[8] the most common method for attaching ethylenediaminetetraacetic acid (EDTA) to macromolecules involves the reaction of EDTA dianhydride with an amine residue on the target molecule. However, in this latter case, the stability constant of the resulting carboxamide-derivatized metal chelate can be lower than that of the parent EDTA. One solution to circumvent this difficulty is to design an EDTA derivative bearing an additional reactive function dedicated to the attachment to the target molecule. Several EDTA–based bifunctional chelating agents have been described, corresponding either to the modification of the ethylenediamino fragment or to the modification at the methylene carbon of one carboxymethyl arm; among them are EDTA derivatives bearing a carboxylic acid,[9] a bromoacetate,[10] an amine,[11] or an isothiocyanobenzyl reactive moiety.[12]

Herein is reported the synthesis, starting from serine, of two nitro derivatives of NTA and EDTA, compounds **1** and **2**, which are modified on one carboxymethyl arm (Figure 1). These molecules are key intermediates for the straightforward synthesis of bifunctional and trifunctional chelating agents.

RESULTS AND DISCUSSION

As outlined in Scheme 1, the NTA derivative **3** was built by double *N*-alkylation of serine *tert*-butyl ester using *tert*-butyl bromoacetate at the reflux of acetonitrile. Under similar conditions, but in the presence of 1.2 equivalent of *tert*-butyl bromoacetate, the product of mono *N*-alkylation **4** was obtained in a 67% yield. This compound was further *N*-alkylated using *N*, *N*-bis[(*tert*-butoxycalbonyl)methyl]-2-bromoethylamine[13] to afford the EDTA derivative **5**. It should be underlined that *tert*-butyl esters were chosen as protective groups

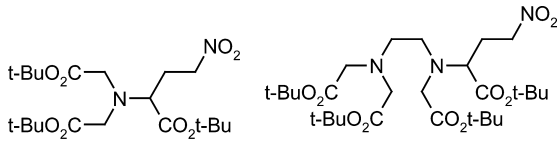
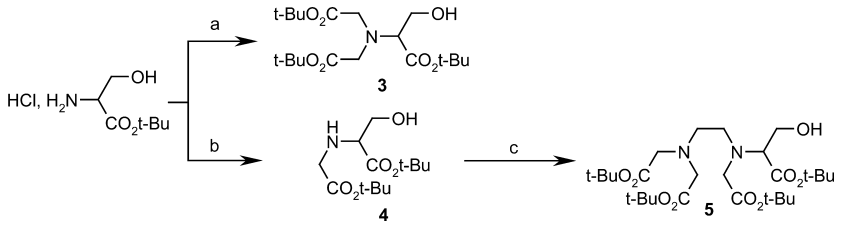


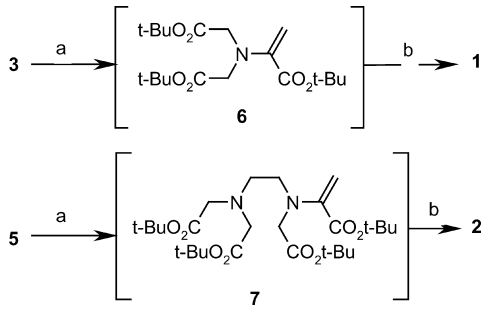
Figure 1. Structure of key compounds **1** and **2**.



Scheme 1. (a) $BrCH_2CO_2t$-Bu (4 equiv), DIPEA (6 equiv), CH_3CN, reflux, 20 h (70%); (b) $BrCH_2CO_2t$-Bu (1.2 equiv), DIPEA (4 equiv), CH_3CN, reflux, 4 h (67%); (c) $Br(CH_2)_2N(CH_2CO_2t\text{-Bu})_2$ (3 equiv), DIPEA (5 equiv), CH_3CN, reflux, 20 h (87%).

for the carboxylate moieties because of the ease of deprotection in the presence of TFA, and to avoid the lactonization that was observed when serine was submitted to *N*-alkylation with methyl bromoacetate.

The nitro derivatives **1** and **2** were obtained by a two-step reaction from the primary alcohols **3** and **5**, as shown in Scheme 2. The use of Mitsunobu reagents, tris(*n*-butyl)phosphine and diethyl azodicarboxylate, allowed the smooth conversion of the serine derivatives **3** and **5** into the didehydroamino acids **6** and **7**, presumably through α-deprotonation.[14] NMR analysis of the crude mixtures obtained after evaporation of the solvent under reduced pressure demonstrated that these reactions proceed very cleanly and in excellent yield; however, the didehydroaminoacids **6** and **7** could not be purified because of their instability on silica gel. Michael additions of nitromethane to **6** and **7** in the presence of fluoride ions afforded the nitro derivatives **1** and **2** in 50% and 64% yields. The addition of nitromethane on several protected dehydroalanines (phthalimido, benzyloxycarbonylamino, acetamido) has been already extensively studied.[15] In the case of **6** and **7**, despite a reduced reactivity resulting from the absence of electron-



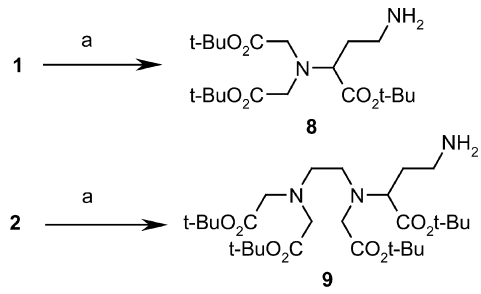
Scheme 2. (a) $(n\text{-Bu})_3P$ (1.5 equiv), DEAD (1.5 equiv), Et_2O, from $-10^{\circ}C$ to rt, 3 h; (b) $n\text{-Bu}_4NF \cdot 3H_2O$ (6 equiv), CH_3NO_2, 50°C, 20 h (**1** 50%, **2** 64%).

withdrawing groups on the amino site of the didehydroaminoacid moiety, smooth additions occurred in the presence of an excess of nitromethane.

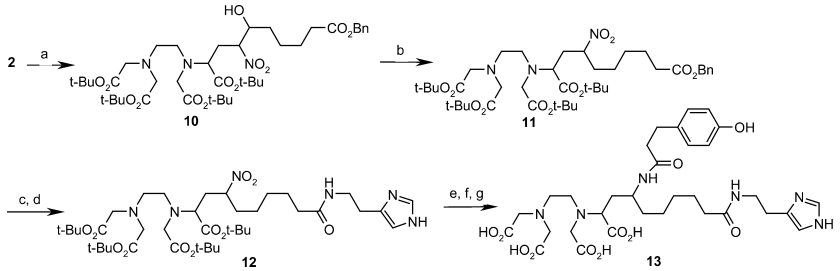
As shown in Scheme 3, the nitro derivatives **1** and **2** are readily reduced by ammonium formate in the presence of palladium on activated carbon to afford the bifunctional chelating agents **8** and **9** in good yields. At this stage the carboxylic acids are quantitatively deprotected by the treatment of **8** or **9** in pure trifluoroacetic acid at room temperature for 6 h. Although the protocols are not emphasized in this report, these bifunctional polyaminocarboxylate chelates can be conjugated to proteins using, for instance, a commercially available bis-*N*-hydroxysuccinimide linker.

To illustrate the preparation of trifunctional chelating agents starting from the nitro derivatives **1** or **2**, the synthesis of such a molecule was investigated (Scheme 4). In the course of our research project a trifunctional molecule was needed that would contain (1) an EDTA moiety to chelate iron, (2) a histamine-like fragment to be recognized by an antihistamine monoclonal antibody, and (3) a Bolton–Hunter fragment allowing the introduction of radioisotopes (compound **13**, Scheme 4). In a first step the EDTA derivative **2** was condensed on benzyl 6-oxohexanoate via an Henry reaction. Among the different sets of conditions or catalysts reported to promote the nitroaldol reaction,[16] the use of neutral alumina,[17] and the *n*-Bu_4NF/triethylamine/*tert*-butyldimethylchlorosilane reagent system were found unacceptably slow or inefficient in that case.[18] However, the adduct **10** was obtained with an excellent yield using one equivalent of *n*-Bu_4NF in THF after 6 h at room temperature. The use of a catalytic amount of *n*-Bu_4NF led to a slow reaction and a moderate yield (for 0.2 equiv, yield was 63% after 2 days at room temperature). The β-nitroalcohol **10** was then converted to the nitro compound **11** by a two-step, one-pot reaction, corresponding to the dehydration of **10** by the use of tris(*n*-butyl)phosphine and diethyl azodicarboxylate. This led to the intermediate nitroalkene and its subsequent reduction in the presence of sodium borohydride.

Hydrogenolysis of the benzyl ester on compound **11** in the presence of palladium on activated carbon under an atmospheric pressure of hydrogen



Scheme 3. (a) Pd/C (50 wt.%), HCO_2NH_4 (40 equiv), MeOH, rt, 1 h (**1** 75%, **2** 80%).



Scheme 4. (a) $HCO(CH_2)_4CO_2Bn$ (1.5 equiv), *n*-$Bu_4NF \cdot 3H_2O$ (1 equiv), THF, rt, 6 h (95%); (b) (*n*-$Bu)_3P$ (2 equiv), DEAD (2 equiv), CH_2Cl_2, 0°C, 1 h, then dilution with EtOH, $NaBH_4$ (6 equiv), 0°C, 1 h (49%); (c) Pd/C (10 wt.%), H_2 (1 atm.), MeOH, rt, 1 h (96%); (d) NHS (1.1 equiv), DCC (1.1 equiv), CH_2Cl_2, rt, 4 h, then histamine (1.3 equiv), DMF, rt, 1.5 h (91%); (e) Pd/C (10 wt.%), HCO_2NH_4 (40 equiv), MeOH, rt, 6 h (77%); (f) Bolton–Hunter reagent (1.2 equiv), TEA (3 equiv), CH_2Cl_2, rt, 20 h (57%); (g) TFA, rt, 6 h (95%).

was carried out quantitatively without reducing the nitro group. Activation of the obtained carboxylic acid as an *N*-hydroxysuccinimidyl ester and subsequent coupling with histamine led to **12** in high yield (Scheme 4). Then, the nitro group was reduced using ammonium formate and palladium on activated carbon, and the corresponding amine was coupled with the Bolton–Hunter reagent. Finally, quantitative removal of the *tert*-butyl ester protective groups by treatment with trifluoroacetic acid at room temperature led to the trifunctional chelating agent **13**.

In conclusion, the synthesis of new nitro derivatives of NTA and EDTA have been reported. These compounds are versatile building blocks for the preparation of bi- or trifunctional chelating agents, taking advantage of the nitro moiety reactivity. This was illustrated by the preparation of a bifunctional metal-chelating agent via the reduction of the nitro group and the design of a trifunctional metal-chelating agents via the coupling with an aldehyde using a Henry reaction.

EXPERIMENTAL

Analytical thin-layer chromatography (TLC) was performed using 0.25-mm silica gel–coated MERCK 60 F_{254} plates. Visualization of the chromatogram was by UV absorbance and ethanolic phosphomolybdic acid. Flash chromatography was performed using compressed air with the indicated solvent system and silica gel 60 (MERCK, 230–400 mesh). ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a BRUKER AC 300 spectrometer. Chemical shifts are reported in parts per million (ppm) on the δ scale from an internal standard. Mass spectra (MS) by chemical ionisation in ammonia

(CI/NH_3) were recorded on a quadripolar FINNIGAN-MAT 4600 spectrometer and by electrospray on an ESI/TOF MARINER spectrometer.

tert-Butyl-2-di(*tert*-butyloxycarbonylmethyl)amino-3-hydroxypropanoate (3)

To a suspension of serine *tert*-butyl ester hydrochloride (600 mg, 3.04 mmol) in acetonitrile (25 mL), di-*iso*-propylethylamine (3.17 mL, 18.23 mmol) and *tert*-butyl bromoacetate (1.79 mL, 12.15 mmol) were added. The mixture was heated under reflux for 20 h. After cooling at room temperature and evaporation of the solvent under reduced pressure, the residue was purified by column chromatography (silica gel, hexane–EtOAc 8 : 2) to afford **3** (826 mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.42 (s, 27H), 3.38–3.55 (m, 6H), 3.67–3.78 (m, 1H), 4.28 (d, $J = 10.3$ Hz, 1H). MS (CI, NH_3): m/z 390 [M + 1].

tert-Butyl-2-(*tert*-butyloxycarbonylmethylamino)-3-hydroxypropanoate (4)

To a suspension of serine *tert*-butyl ester hydrochloride (3.58 g, 18.14 mmol) in acetonitrile (150 mL), di-*iso*-propylethylamine (11.42 mL, 72.55 mmol) and *tert*-butyl bromoacetate (3.21 mL, 21.76 mmol) were added. The mixture was heated under reflux for 4 h. After cooling at room temperature and evaporation of the solvent under reduced pressure, the residue was purified by column chromatography (silica gel, hexane–EtOAc 5 : 5) to afford **4** (3.33 g, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.46 (s, 9H), 1.50 (s, 9H), 3.25 (dd ABX-system, $J = 4.3$ and 6.1 Hz, 1H), 3.27 and 3.39 (2d AB-system, $J = 17.1$ Hz, 2H), 3.60 (dd ABX-system, $J = 6.1$ and 11.0 Hz, 1H), 3.72 (dd ABX-system, $J = 4.3$ and 11.0 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 27.8, 49.5, 62.3, 62.6, 81.3, 81.6, 171.2, 171.3. MS (CI, NH_3): m/z 276 [M + 1].

tert-Butyl-2-*tert*-butyloxycarbonylmethyl[2-di(*tert*-butyloxycarbonylmethyl)aminoethyl]amino-3-hydroxypropanoate (5)

To a solution of **4** (300 mg, 1.09 mmol) in acetonitrile (10 mL) di-*iso*-propylethylamine (0.87 mL, 5.45 mmol) and *N*,*N*-bis[(*tert*-butoxycalbonyl)methyl]-2-bromoethylamine (1.15 g, 3.27 mmol)[13] were added. The mixture was heated under reflux for 20 h. After cooling at room temperature and evaporation of the solvent under reduced pressure, the residue was purified by column chromatography (silica gel, hexane–EtOAc 7 : 3) to afford **5** (520 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.43 (s, 36H), 2.74–2.98 (m, 4H),

3.30–3.55 (m, 8H), 3.65–3.80 (m, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 27.7, 27.8, 27.9, 52.0, 53.2, 53.7, 55.9, 59.7, 67.2, 80.7, 81.1, 81.2, 170.2, 170.4, 172.1. MS (CI, NH_3): m/z 547 [M + 1].

Typical Procedure for the Synthesis of *tert*-Butyl-2-di(*tert*-butyloxycarbonylmethyl)amino-4-nitrobutanoate (1) and *tert*-Butyl-2-*tert*-butyloxycarbonylmethyl[2-di(*tert*-butyloxycarbonylmethyl)aminoethyl]amino-4-nitrobutanoate (2)

To a solution of **5** (150 mg, 0.27 mmol) in diethyl ether (6 mL) under argon and at -10°C, tris(*n*butyl)phosphine (100 μL, 0.41 mmol) and diethyl azodicarboxylate (65 μL, 0.41 mmol) were added. The mixture was stirred for 3 h, while the temperature was allowed to reach 20°C. After evaporation of the solvent under reduced pressure, the crude material was diluted with nitromethane (8 mL), and tributylammonium fluoride trihydrate (520 mg, 1.65 mmol) was added. The mixture was stirred at room temperature for 1 h, and at 50°C overnight. After cooling at room temperature and evaporation of the solvent under reduced pressure, the residue was purified by column chromatography (silica gel, hexane–EtOAc 8 : 2) to afford **2** (103 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.41 (s, 27H), 1.43 (s, 9H), 2.08–2.40 (m, 2H), 2.67–2.93 (m, 4H), 3.21 (d AB-system, $J = 17.1$ Hz, 1H), 3.33 (d AB-system, $J = 17.1$ Hz, 1H), 3.37 (d AB-system, $J = 17.7$ Hz, 2H), 3.45 (d AB-system, $J = 17.7$ Hz, 2H), 3.62 (dd ABX-system, $J = 4.3$ and 11.6 Hz, 1H), 4.60–4.88 (m, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 27.7, 27.8, 28.0, 50.6, 51.2, 53.8, 55.6, 60.5, 72.1, 80.7, 80.8, 81.5, 170.3, 170.6. HRMS: m/z calcd.: 590.3652; found: 590.3657 [M + 1]. Analytical data for compound 1: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.42 (s, 18H), 1.45 (s, 9H), 2.10–2.45 (m, 2H), 3.34 (d AB-system, $J = 17.1$ Hz, 2H), 3.37 (d AB-system, $J = 17.1$ Hz, 2H), 3.47 (dd ABX-system, $J = 4.3$ and 11.6 Hz, 1H), 4.68–4.90 (m, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 27.3, 27.8, 27.9, 54.0, 61.9, 71.9, 80.9, 81.8, 169.9, 170.4. HRMS: m/z calcd.: 433.2550; found: 433.2547 [M + 1].

Typical Procedure for the Synthesis of *tert*-Butyl-4-amino-2-di(*tert*-butyloxycarbonylmethyl)aminobutanoate (8) and *tert*-Butyl-4-amino-2-*tert*-butyloxycarbonylmethyl[2-di(*tert*-butyloxycarbonylmethyl)aminoethyl]aminobutanoate (9)

To a solution of **2** (100 mg, 0.17 mmol) in methanol (3 mL), palladium on activated carbon (20 mg) and ammonium formate (428 mg, 40 eq) were added in four portions every hour. The catalyst was filtered and washed with methanol. The filtrate was evaporated under reduced pressure. The residue was diluted with methylene chloride and washed with a saturated

solution of sodium hydrogenocarbonate. The organic phase was dried over magnesium sulfate and evaporated under reduced pressure to afford **9** (76 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30–1.50 (m, 36H), 1.80–2.10 (m, 2H), 2.58–2.90 (m, 4H), 3.18–3.50 (m, 9H), 3.67–3.74 (m, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 25.6, 27.7, 27.8, 27.9, 49.7, 50.1, 55.0, 60.8, 81.4, 81.7, 82.0, 170.4, 172.0. MS (CI, NH_3): m/z 560 [M + 1]. Analytical data for compound 8: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.41 (bs, 27H), 1.90–2.30 (m, 2H), 3.05–3.60 (m, 7H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 25.0, 27.8, 27.9, 38.6, 53.7, 64.1, 81.9, 82.0, 169.6, 170.6. MS (CI, NH_3): m/z 403 [M + 1].

Procedure for the Synthesis of 10

To a solution of **2** (800 mg, 1.36 mmol) in THF (20 mL), benzyl 6-oxohexanoate (400 mg, 2.04 mmol) and tributylammonium fluoride trihydrate (428 mg, 1.36 mmol) were added. The mixture was stirred for 6 h at room temperature. After evaporation of the solvent under reduced pressure, the crude material was diluted with methylenechloride and washed twice with water and once with brine. The organic phase was dried over magnesium sulfate and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane–EtOAc 8 : 2) to afford **10** (1.5 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90–1.80 (m, 40H), 1.92–2.50 (m, 6H), 2.55–2.95 (m, 4H), 3.00–3.78 (m, 7H), 3.80–4.25 (m, 1H), 4.85–5.35 (m, 3H), 7.20–7.45 (m, 5H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 24.4, 25.0, 27.8, 27.9, 28.0, 29.3, 29.9, 31.4, 32.5, 33.9, 51.3, 52.0, 54.0, 55.0, 55.7, 60.8, 61.2, 65.9, 70.5, 71.4, 72.2, 80.9, 81.0, 81.6, 87.2, 88.2, 127.9, 128.3, 135.8, 170.5, 170.7, 173.1. MS (CI, NH_3): m/z 810 [M + 1]. MS (TOF): m/z 832 [M + 23].

Procedure for the Synthesis of 11

To a solution of **10** (270 mg, 0.33 mmol) in methylene chloride (2 mL) were added, under argon and at -15°C, tris(*n*butyl)phosphine (165 μL, 0.66 mmol) and diethyl azodicarboxylate (105 μL, 0.66 mmol). The mixture was stirred for 1 h at 0°C, then cooled at -5°C, diluted with ethanol (7 mL), and sobium borohydride (75 mg, 1.99 mmol) was added. The mixture was stirred for 1 h at 0°C, and quenched by addition of water. The solution was extracted twice with ethyl acetate, the organic phases were dried over magnesium sulfate and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane–EtOAc 8 : 2) to afford **11** (126 mg, 49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23–2.25 (m, 44H), 2.22–2.43 (m, 4H), 2.67–2.98 (m, 4H), 3.12–3.60 (m, 7H), 4.82 (quint., $J = 7.2$ Hz, 0.5H), 5.00–5.34 (m, 2.5H), 7.28–7.43 (sl, 5H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 24.3, 25.0, 27.8, 27.9,

28.3, 33.1, 33.3, 33.4, 33.8, 34.1, 50.1, 52.0, 52.2, 52.5, 53.5, 53.9, 55.7, 55.8, 60.6, 61.7, 65.8, 80.6, 81.5, 84.4, 85.3, 127.9, 128.3, 135.8, 170.3, 170.7, 170.8, 170.9, 173.0. MS (TOF): m/z 794 [M + 1].

Procedure for the Synthesis of 12

To a solution of **11** (112 mg, 0.14 mmol) in methanol (3 mL) was added palladium on activated carbon (10 mg). The mixture was stirred for 1 h under hydrogen (1 atm). The catalyst was filtered and washed with methanol. The filtrate was evaporated under reduced pressure to afford the corresponding benzyl-deprotected carboxylic acid (96 mg, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.20–2.15 (m, 44H), 2.22–2.42 (m, 4H), 2.62–3.05 (m, 4H), 3.18–3.80 (m, 7H), 4.80–4.92 (m, 0.5H), 4.98–5.12 (m, 0.5H). MS (TOF): m/z 704 [M + 1]. To the solution of this compound in methylene chloride (2 mL), *N*-hydroxysuccinimide (18 mg, 0.15 mmol) and dicyclocarbodiimide (31 mg, 0.15 mmol) were added. The mixture was stirred for 4 h and filtered. The filtrate was evaporated under reduced pressure. The residue obtained was diluted with DMF (2 mL), and histamine (20 mg, 0.17 mmol) was added. The mixture was stirred for 1.5 h, and then the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, methylene chloride–methanol 9 : 1) to afford **12** (99 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.12–1.30 (m, 4H), 1.40 (sl, 36H), 1.45–2.02 (m, 5H), 2.10 (t, $J = 7.3$ Hz, 2H), 2.20–2.35 (m, 1H), 2.62–2.92 (m, 6H), 3.10–3.50 (m, 9H), 4.77 (quint., $J = 6.7$ Hz, 0.5H), 4.93–5.08 (m, 0.5H), 6.68 (t, $J = 6.5$ Hz, 1H), 6.76 (s, 1H), 7.54 (s, 1H). MS (TOF): m/z 797 [M + 1].

Procedure for the Synthesis of 13

To a solution of **12** (83 mg, 0.10 mmol) in methanol (3 mL) were added palladium on activated carbon (20 mg) and ammonium formate (264 mg, 40 eq) in four portions every 90 min. The catalyst was filtered and washed with methanol. The filtrate was evaporated under reduced pressure. The residue was diluted with methylene chloride and washed with a saturated solution of sodium hydrogenocarbonate. The organic phase was dried over magnesium sulfate and evaporated under reduced pressure to afford the expected amine (62 mg, 77%) as the product of the nitro function reduction. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.00–1.93 (m, 46H), 2.11 (t, $J = 7.3$ Hz), 2.58–2.90 (m, 6H), 3.00–3.80 (m, 10H), 6.74 (s, 1H), 7.45 (s, 1H). MS (TOF): m/z 767 [M + 1]. To the solution of the previous compound in methylene chloride (2 mL), triethylamine (35 μL, 0.24 mmol) and the Bolton–Hunter reagent (26 mg, 0.10 mmol) were added. The mixture was stirred for 20 h, and then diluted with methylene chloride and washed with

a saturated solution of sodium hydrogenocarbonate. The organic phase was dried over magnesium sulfate and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, methylene chloride–methanol 9 : 1) to afford the expected coupling adduct (42 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.25–1.90 (m, 46H), 2.03 (t, $J = 7.3$ Hz, 2H), 2.42–2.60 (m, 2H), 2.62–3.00 (m, 8H), 3.12–3.92 (m, 10H), 6.68–6.82 (m, 3H), 7.02 (d, $J = 6.7$, 1H), 7.04 (d, $J = 8.5$, 1H), 7.53 (s, 1H). MS (TOF): m/z 937 [M + 23]. The solution of the previous compound in trifluoroacetic acid (3 mL) was stirred for 6 h. Diethyl ether was added, and the precipitate was filtered, washed with diethyl ether, and dried under vacuum to afford 13-(tris-trifluoroacetate salt) (40 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.20–2.00 (m, 10H), 2.02–2.15 (m, 2H), 2.38–2.52 (m, 2H), 2.70–3.20 (m, 8H), 2.35–2.70 (m, 4H), 3.80–4.48 (m, 6H), 6.62 (d, $J = 7.9$ Hz, 2H), 6.97 (d, $J = 7.9$ Hz, 1H), 6.99 (d, $J = 7.9$ Hz, 1H), 7.28 (s, 1H), 8.75 (s, 1H). MS (TOF): m/z 691 [M + 1].

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