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A Convenient Synthesis for HBED-CC-tris(*tert*-butyl ester)

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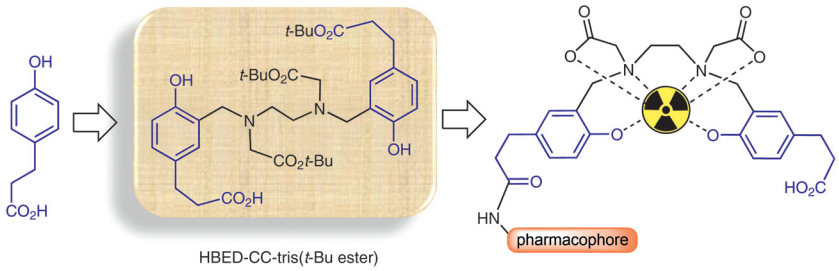

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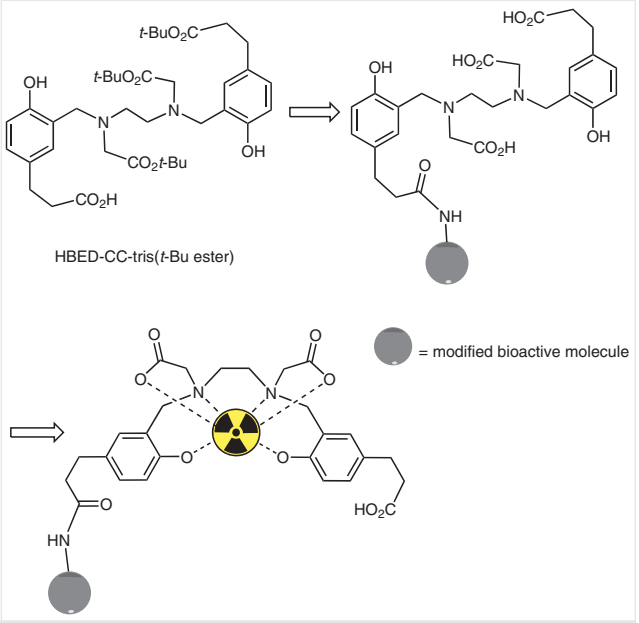
Abstract HBED-CC is a bifunctional complexing agent that, at ambient temperature, tightly chelates the trivalent radiometal ^{68}Ga ($T_{1/2}$ = 68 min). This complexing agent has attracted a lot of interest in tumor imaging applications. Depending on the chemical structure, different HBED-CC variants may be employed as radiolabeling precursor for the synthesis of desired radiopharmaceuticals. In this context, HBED-CC-tris(tert-butyl ester) is the only known monovalent variant of HBED-CC which is used for the synthesis of non-symmetric HBED-CC-based radiopharmaceuticals. Commercial HBED-CC-tris(*tert*-butyl ester) is very expensive, with limited availability. Nevertheless, no synthetic procedure for this useful product has been reported to date. This work introduces a convenient and comparatively cost-efficient method for the preparation of HBED-CC-tris(*tert*-butyl ester).

Key words HBED-CC, bifunctional chelator, hexadentate ligand, synthesis, radiotracer



Scheme 1 Preparation of monovalent radiopharmaceuticals, starting with HBED-CC-tris(*tert*-butyl ester)

Radiopharmaceuticals are essential for cancer diagnosis as well as therapy in nuclear medicine.[1–3] To date, a variety of radiopharmaceuticals with different structures and functional groups have been introduced.[4,5] Among them, radiometal-based radiopharmaceuticals bearing the complexing agent *N*,*N*′-bis[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-*N*,*N*′-diacetic acid (HBED-CC) have been drawing the attention of many research groups over the past decade.[6–13] HBED-CC is a bifunctional hexadentate ligand with an N_2O_4 donor set that forms strong complexes with gallium, in particular in this context, its radioactive isotopes, such as the positron emitter gallium-68.[14] Therefore, ^{68}Ga-labelled HBED-CC-based radiopharmaceuticals have become popular in recent years for noninvasive imaging by means of positron-emission-tomography/computer tomography (PET/CT) due to their particular nuclear physical and at the same time pharmacokinetic characteristics for highly sensitive and specific diagnosis of cancer (i.e., ^{68}Ga[Ga]DKFZ-11, prostate cancer diagnosis) and other diseases. Hence, syntheses of HBED-CC variants and their corresponding radiometal-based radiopharmaceuticals have been developed in recent years.[15–20] In this context, HBED-CC-tris(*tert*-butyl ester)[21] is the only known monovalent variant of HBED-CC. This compound is employed as a precursor for synthesis of HBED-CC-based radiopharmaceuticals with non-symmetric structures (Scheme 1).[15] In general, preparation of HBED-CC-based radiopharmaceuticals from the HBED-CC-tris(*tert*-butyl ester) precursor is divided into two main steps, namely bioconjugation and subse-

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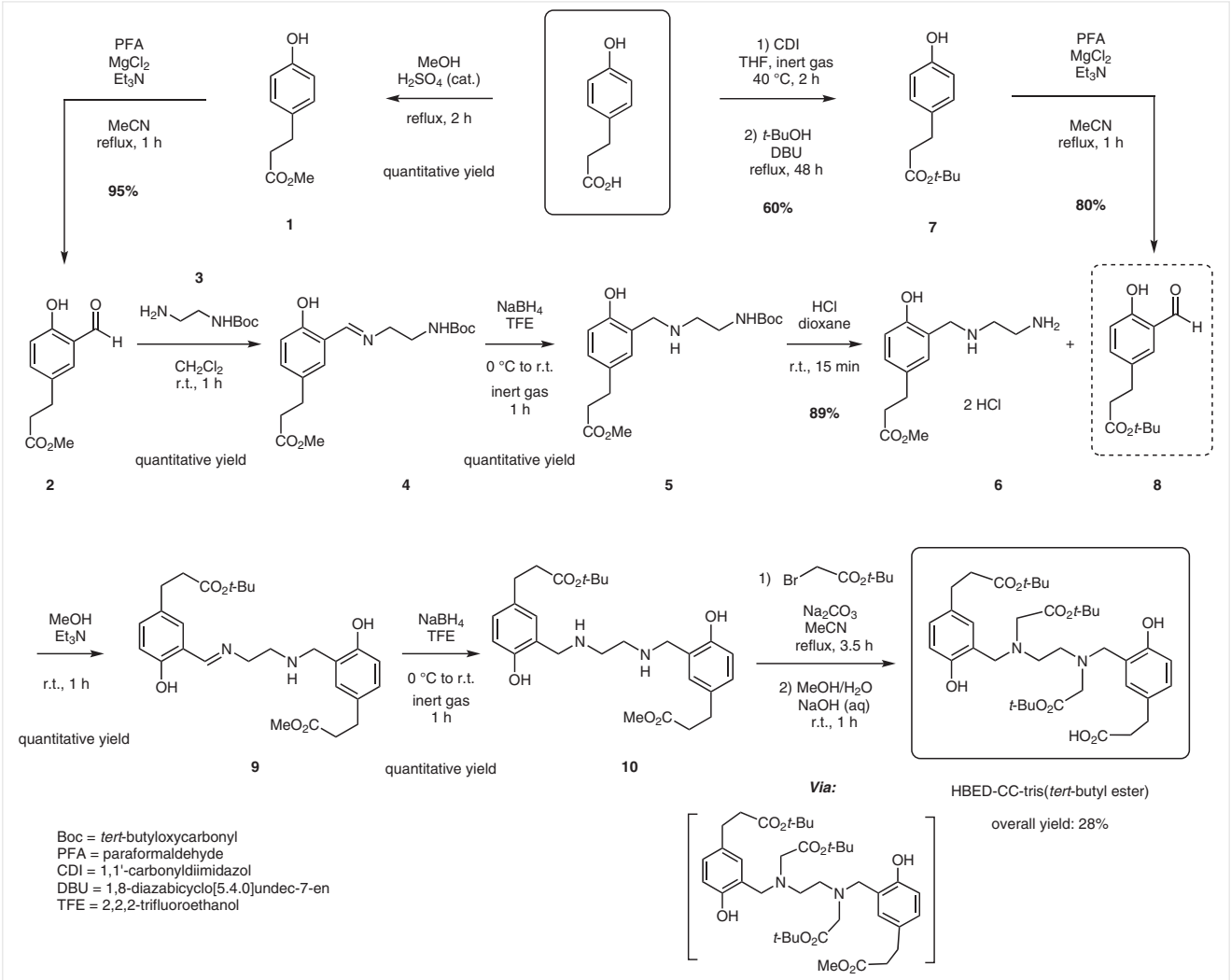
quent radiolabeling. Initially, the HBED-CC triester precursor binds a modified bioactive molecule (e.g., a peptide, inhibitor). In the next step, the *tert*-butyl groups have to be removed to yield the complete set of coordinating sites ready for radiolabeling with radiogallium, which results in very strong complexes.[22]

However, commercial HBED-CC-tris(*tert*-butyl ester) is very expensive and has limited availability. To our knowledge, no synthetic method and characterization data for this compound have yet been reported. Therefore, there is a need to find a suitable and efficient method to prepare this very useful complexing agent. The current work presents a new and convenient synthetic procedure for HBED-CC-tris(*tert*-butyl ester), together with full structural characterization of this product and its intermediates (Scheme 2).

As illustrated in Scheme 2, the synthetic pathway starts with 4-hydroxyhydrocinnamic acid as the first commercially available reactant. Esterification of the carboxylic acid group of this gives product **1**.[23] This product is converted into aldehyde **2** through *ortho*-formylation.[24] Then, aldehyde **2** is transformed into amine **5** by reductive amination in the 2,2,2-trifluoroethanol (pK_a 12.4)[25] solution. This solvent adjusts the pH to 5–6, which is ideal for reduction of the imine intermediate.[26] Use of other common protic solvents, such as methanol, for this reaction causes incomplete reaction and increases the number of by-products. Subsequently, removal of the *tert*-butyl dicarbonate (Boc) group provides salt **6**. This salt is then used for the reductive amination of **8** to provide amine **10**. Intermediate **8** is prepared from ester **7** through *ortho*-formylation.[24] Finally, compound **10** is alkylated, followed by hydrolysis to yield HBED-CC-tris(*tert*-butyl ester) as the final product (Scheme 2).

In conclusion, we have developed a convenient and cost-efficient procedure for the synthesis of HBED-CC-tris(*tert*-butyl ester).[27,28] All the commercially available chemicals



Scheme 2 Synthesis of HBED-CC-tris(*tert*-butyl ester)

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used in our synthetic protocol are relatively low-priced. Furthermore, most reaction steps are short and easy to perform, and also involve simple, fast and cost-efficient purification methods.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0036-1591950.

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(27) Preparation of the known compounds are described in the Supporting Information.
(28) **Synthesis of Methyl 3-{3-[({2-[(*tert*- Butoxycarbonyl)amino] ethyl}imino)methyl]-4-hydroxyphenyl}propanoate (4)**
To a solution of methyl 3-(3-formyl-4-hydroxyphenyl)propanoate (**2**) (3.00 g, 14.41 mmol, 1.0 equiv) in anhydrous dichloromethane (10 mL) was added *tert*-butyl (2-aminoethyl)carbamate (**3**) (2.54 g, 15.85 mmol, 1.1 equiv). The mixture was stirred at room temperature for 1 h, and then diluted with dichloromethane (40 mL). The reaction mixture was then washed with aq. sodium bisulfite (0.5 M, 50 mL). The product residue in the aqueous phase was recovered by washing four times with dichloromethane. Then, the combined organic phases were dried over sodium sulfate, filtered and the solvent was removed under reduced pressure to give product **4** as an orange oil (quantitative yield). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 8.32 (s, 1 H, CC*H*N), 7.15 (dd, $^3J_{H-H}$ = 8.4 Hz, $^4J_{H-H}$ = 2.2 Hz, 1 H, CHC*H*CCH), 7.09 (d, $^4J_{H-H}$ = 2.2 Hz, 1 H, CC*H*C), 6.90 (d, $^3J_{H-H}$ = 8.4 Hz, 1 H, C*H*CHCCH), 4.72 (br s, 1 H, NH), 3.70 (t, $^3J_{H-H}$ = 5.7 Hz, 2 H, CH_2CH_2NHBoc), 3.66 (s, 3 H, OCH_3), 3.45 (m, 2 H, CH_2CH_2NHBoc), 2.90 (t, $^3J_{H-H}$ = 7.7 Hz, 2 H, $CH_2CH_2CO_2$), 2.60 (t, $^3J_{H-H}$ = 7.7 Hz, 2 H, $CH_2CH_2CO_2$), 1.43 (s, 9 H, $C(CH_3)_3$) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ = 173.40 (CO_2CH_3), 166.59 (COH), 159.57 (CCHN), 155.98 ($^{Boc}CO_2$), 132.65 (CHCHCCH), 131.10 (CHCHCCH), 130.81 (CCHC), 118.60 (CCHN), 117.24 (CHCHCCH), 79.71 ($^{Boc}CO_2CCH_3$), 59.57 (CH_2CH_2NHBoc), 51.78 (CO_2CH_3), 41.37 (CH_2CH_2NHBoc), 36.00 ($CH_2CH_2CO_2$), 30.07 ($CH_2CH_2CO_2$), 28.51 ($C(CH_3)_3$). HRMS (ESI+, CH_2-

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Cl_2/MeOH): *m*/*z* calcd for $C_{18}H_{27}N_2O_5$: 351.1914; found: 351.1914. HRMS (ESI-, CH_2Cl_2/MeOH): *m*/*z* calcd for $C_{18}H_{25}N_2O_5$: 349.1769; found: 349.1769.

Synthesis of Methyl 3-{3-[({2-[(*tert*-Butoxycarbonyl)amino] ethyl}amino)methyl]-4-hydroxyphenyl}propanoate (5)

A solution of compound **4** (4.90 g, 14.00 mmol, 1.0 equiv) in 2,2,2-trifluoroethanol (60 mL) was cooled in an ice-bath. Sodium borohydride (1.32 g, 35 mmol, 2.5 equiv) was added in portions and then the reaction mixture was allowed to warm to room temperature. After being stirred under an inert atmosphere at room temperature for one hour, the reaction was quenched with water (50 mL). The product was extracted with dichloromethane and the organic layer was dried over sodium sulfate and filtered. By removal of the solvent under reduced pressure compound **5** was obtained as a colorless oil in quantitative yield. This product was almost pure and used in the next step without further purification. However, for spectroscopic characterization it was purified through HPLC. ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 6.98 (dd, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 2.2 Hz, 1 H, CHC*H*CCH), 6.81 (d, $^4J_{H-H}$ = 2.2 Hz, 1 H, CCHC), 6.74 (d, $^3J_{H-H}$ = 8.4 Hz, 1 H, C*H*CHCCH), 4.75 (br s, 1 H, CH_2NHCH_2), 3.97 (s, 2 H, CCH_2NH), 3.66 (s, 3 H, OCH_3), 3.28 (m, 2 H, CH_2NHBoc), 2.83 (t, $^3J_{H-H}$ = 7.7 Hz, 2 H, $CH_2CH_2CO_2$), 2.78 (t, $^3J_{H-H}$ = 5.7 Hz, 2 H, CH_2CH_2NHBoc), 2.56 (t, $^3J_{H-H}$ = 7.7 Hz, 2 H, $CH_2CH_2CO_2$), 1.44 (s, 9 H, $C(CH_3)_3$). $^{13}C\{^1H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ = 173.62 (CO_2CH_3), 156.51 ($^{Boc}CO_2$), 156.33 (COH), 131.16 (CHCHCCH), 128.63 (CCHC), 128.47 (CHCHCCH), 122.27 (CCH_2NH), 116.52 (CHCHCCH), 79.78 ($^{Boc}CO_2CCH_3$), 52.48 (CCH_2NH), 51.70 (CO_2CH_3), 48.55 (CH_2CH_2NHBoc), 40.16 (CH_2NHBoc), 36.20 ($CH_2CH_2CO_2$), 30.24 ($CH_2CH_2CO_2$), 28.50 ($C(CH_3)_3$). HRMS (ESI+, CH_2Cl_2/MeOH): *m*/*z* calcd for $C_{18}H_{29}N_2O_5$: 353.2071; found: 353.2071. HRMS (ESI-, CH_2Cl_2/MeOH): *m*/*z* calcd for $C_{18}H_{27}N_2O_5$: 351.1925; found: 351.1925.

Synthesis of [Methyl 3-(3-{[(2- Aminoethyl)amino]methyl}-4-hydroxyphenyl)propanoate] Dihydrochloride (6)

Compound **5** (4.90 g, 13.90 mmol) was dissolved in 4 M HCl in dioxane (20 mL). After being stirred at room temperature for 15 min, diethyl ether (40 mL) was added and the reaction mixture stirred for another 5 min. The white precipitate was filtered, washed with diethyl ether and dried under vacuum to give salt **6** (4.04 g, 12.37 mmol, 89%). Mp 190–191 °C. ^{1}H NMR (400.13 MHz, D_2O, 25 °C): δ = 7.24 (m, 2 H, CHC*H*CC*H*), 6.94 (d, $^3J_{H-H}$ = 9.0 Hz, 1 H, C*H*CHCCH), 4.31 (s, 2 H, $^{Bn}CH_2$), 3.66 (s, 3 H, OCH_3), 3.45 (m, 4 H, CH_2CH_2NH), 2.89 (t, $^3J_{H-H}$ = 7.3 Hz, 2 H, $CH_2CH_2CO_2$), 2.70 (t, $^3J_{H-H}$ = 7.3 Hz, 2 H, $CH_2CH_2CO_2$). $^{13}C\{^1H\}$ NMR (100.61 MHz, D_2O, 25 °C): δ = 176.50 (CO_2CH_3), 153.48 (COH), 132.68 (CHCHCCH), 131.49 (CCHC), 131.42 (CHCHCCH), 116.80 (CCH_2N), 115.70 (CHCHCCH), 52.16 (CO_2CH_3),47.42 ($^{Bn}CH_2$), 43.48 ($ArCH_2NHCH_2$), 35.45 (CH_2NH_2), 35.40 ($CH_2CH_2CO_2$), 29.25 ($CH_2CH_2CO_2$) ppm. Anal. Calcd. (included 3.56% water; hydroscopic salt) C: 46.30, H: 6.97, N: 8.31; found C: 46.46, H: 6.88, N: 8.13.

Synthesis of *tert*-Butyl 3-(4-Hydroxy-3-{[(2-{[2-hydroxy-5-(3-methoxy-3-oxopropyl)benzyl]amino}ethyl)imino] methyl}phenyl)propanoate (9)

Dry triethylamine (1.51 g, 14.88 mmol, 3 equiv) was added to a solution of compound **6** (1.77 g, 5.46 mmol, 1.1 equiv) in absolute methanol (25 mL). To this mixture was added aldehyde **8** (1.24 g, 4.96 mmol, 1 equiv) and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with dichloromethane (50 mL) and washed with sodium bicarbonate solution (0.5 M, 50 mL). The product residue was recovered from the aqueous phase by washing four times with dichloromethane. The combined organic phase was dried over sodium sulfate, filtered and the solvent was removed under reduced pressure to give product **9** as orange solid in quantitative yield. Mp 77–79 °C. ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 8.35 (s, 1 H, CC*H*N), 7.15 (dd, $^3J_{H-H}$ = 8.4 Hz, $^4J_{H-H}$ = 2.2 Hz, 1 H, CHC*H*C-$(CH_2)_2CO_2Me$), 7.08 (d, $^4J_{H-H}$ = 2.2 Hz, 1 H, CC*H*C-$(CH_2)_2CO_2Me$), 6.98 (dd, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 2.1 Hz, 1 H, CHC*H*C-$(CH_2)_2CO_2t$-Bu), 6.88 (d, $^3J_{H-H}$ = 8.4 Hz, 1 H, C*H*CHC-$(CH_2)_2CO_2Me$), 6.81 (d, $^4J_{H-H}$ = 2.1 Hz, 1 H, CC*H*C-$(CH_2)_2CO_2t$-Bu), 6.74 (d, $^3J_{H-H}$ = 8.2 Hz, 1 H, C*H*CHC-$(CH_2)_2CO_2t$-Bu), 3.98 (s, 2 H, $^{Bn}CH_2$), 3.75 (t, $^3J_{H-H}$ = 5.3 Hz, 2 H, CH_2CH_2NH), 3.65 (s, 3 H, OCH_3), 3.00 (t, $^3J_{H-H}$ = 5.3 Hz, 2 H, CH_2CH_2NH), 2.83 (m, 4 H, $CH_2CH_2CO_2Me$ and $CH_2CH_2CO_2t$-Bu), 2.56 (t, $^3J_{H-H}$ = 7.8 Hz, 2 H, $CH_2CH_2CO_2Me$), 2.50 (t, $^3J_{H-H}$ = 7.6 Hz, 2 H, $CH_2CH_2CO_2t$-Bu), 1.40 (s, 9 H, $C(CH_3)_3$). $^{13}C\{^1H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ = 173.58 (CO_2CH_3), 172.28 (CO_2t-Bu), 166.76 (CCHN), 159.31 ($C(OH)CCH_{(imine)}$), 156.53 ($C(OH)CCH_2$), 132.82 (CHCHC-$(CH_2)_2CO_2Me$), 131.17 (C-$(CH_2)_2CO_2Me$ and C-$(CH_2)_2CO_2t$-Bu), 131.15 (CCHC-$(CH_2)_2CO_2Me$), 128.60 (CCHC-$(CH_2)_2CO_2t$-Bu), 128.45 (CHCHC-$(CH_2)_2CO_2t$-Bu), 122.17 (CCH_2NH), 118.4 (CCHN), 117.24 (CHCHC-$(CH_2)_2$-CO_2t-Bu), 116.51 (CHCHC-$(CH_2)_2CO_2Me$), 80.52 (CO_2CCH_3), 59.31 (CH_2CH_2NH), 52.53 ($^{Bn}CH_2$), 51.67 (CO_2CH_3), 48.60 (CH_2CH_2NH), 37.33 ($CH_2CH_2CO_2t$-Bu), 36.18 ($CH_2CH_2CO_2Me$), 30.22 ($CH_2CH_2CO_2Me$), 30.19 ($CH_2CH_2CO_2t$-Bu), 28.18 ($C(CH_3)_3$). HRMS (ESI+, CH_2Cl_2/MeOH): *m*/*z* calcd for $C_{27}H_{37}N_2O_6$: 485.2646; found: 485.2649; $C_{27}H_{36}N_2NaO_6$: 507.2471; found: 507.2474. HRMS (ESI-, CH_2Cl_2/MeOH): *m*/*z* calcd for $C_{27}H_{35}N_2O_6$: 483.2501; found: 483.2500.

Synthesis of *tert*-Butyl 3-(4-hydroxy-3-{[(2-{[2-hydroxy-5-(3-methoxy-3-oxopropyl)ben-zyl]amino}ethyl)amino]methyl} phenyl)propanoate (10)

A solution of compound **9** (2.33 g, 4.80 mmol, 1.0 equiv) in 2,2,2-trifluoroethanol (50 mL) was cooled in an ice-bath. Sodium borohydride (0.45 g, 12 mmol, 2.5 equiv) was added in portions to this solution, and then the reaction mixture was allowed to warm to room temperature. After being stirred under inert gas atmosphere at room temperature for one hour, the reaction was quenched with water (50 mL). The product was extracted with dichloromethane and the organic layer was dried over sodium sulfate and filtered. By removal of the solvent under reduced pressure white solid **10** was obtained in quantitative yield. Mp 74–76 °C. ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 6.98 (d, $^3J_{H-H}$ = 8.2 Hz, 2 H, C*H*CHC-$(CH_2)_2CO_2Me$ and C*H*CHC-$(CH_2)_2CO_2t$-Bu), 6.80 (br s, 2 H, CC*H*C-$(CH_2)_2CO_2Me$ and CC*H*C-$(CH_2)_2CO_2t$-Bu), 6.73 (dd, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 2.2 Hz, 2 H, CHC*H*C-$(CH_2)_2CO_2Me$ and CHC*H*C-$(CH_2)_2CO_2t$-Bu), 3.94 (s, 2 H, $^{Bn}CH_2$), 3.65 (s, 3 H, OCH_3), 2.81 (m, 8 H, $NH(CH_2)_2NH$, CH_2CH_2-CO_2Me and $CH_2CH_2CO_2t$-Bu), 2.56 (t, $^3J_{H-H}$ = 7.5 Hz, 2 H, CH_2CH_2-CO_2Me), 2.50 (t, $^3J_{H-H}$ = 7.8 Hz, 2 H, $CH_2CH_2CO_2t$-Bu), 1.41 (s, 9 H, $C(CH_3)_3$). $^{13}C\{^1H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ = 173.60 (CO_2CH_3), 172.56 (CO_2t-Bu), 156.38 (COH), 156.53 (COH), 131.49 (C-$(CH_2)_2CO_2Me$), 131.20 (C-$(CH_2)_2CO_2t$-Bu), 128.70 (CCHC-$(CH_2)_2CO_2Me$), 128.62 (CCHC-$(CH_2)_2CO_2t$-Bu), 128.48 (CHCHC-$(CH_2)_2CO_2Me$), 128.44 (CHCHC-$(CH_2)_2CO_2t$-Bu), 122.22 (CCHC-$(CH_2)_2CO_2Me$), 122.06 (CCHC-$(CH_2)_2CO_2t$-Bu), 116.46 (CHCHC-$(CH_2)_2CO_2Me$), 116.34 (CHCHC-$(CH_2)_2CO_2t$-Bu), 80.37 (CO_2CCH_3), 52.70 ($^{Bn}CH_2$), 51.68 (CO_2CH_3), 47.97 (CH_2CH_2NH-$ArCO_2Me$), 47.93 (CH_2CH_2NH-$ArCO_2t$-Bu), 37.55 ($CH_2CH_2CO_2t$-Bu), 36.16 ($CH_2CH_2CO_2Me$), 30.38 ($CH_2CH_2CO_2t$-Bu), 30.20 ($CH_2CH_2CO_2Me$), 28.18 ($C(CH_3)_3$). HRMS (ESI+, CH_2Cl_2/MeOH): *m*/*z* calcd for $C_{27}H_{39}N_2O_6$: 487.2803; found: 487.2816. HRMS (ESI-, CH_2Cl_2/MeOH): *m*/*z* calcd for $C_{27}H_{37}N_2O_6$: 485.2657;

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found: 485.2657.

Synthesis of 3-(3-{[(2-{[5-(2-*tert*-Butoxycarbonylethyl)-2-hydroxybenzyl]-*tert*-butoxycarbonylmethylamino}ethyl)-*tert*-butoxycarbonylmethylamino]methyl}-4-hydroxyphenyl)propionic Acid [HBED-CC-tris(*tert*-butyl ester)]

Compound **10** (1.10 g, 2.26 mmol, 1 equiv) and anhydrous sodium carbonate (0.96 g, 9.06 mmol, 4 equiv) were suspended in anhydrous acetonitrile (25 mL). To this mixture was added *tert*-butyl 2-bromoacetate (0.93 g, 4.76 mmol, 2.1 equiv). The reaction mixture stirred for 3.5 h under reflux conditions. It was cooled to room temperature, filtered and the solvent was removed under reduced pressure. Then the residue was dissolved in methanol (15 mL), followed by dilution with water (10 mL). To this mixture was added sodium hydroxide solution (4 M, 5 mL) slowly. After being stirred for 1 h, the reaction mixture was cooled in an ice-bath and the pH was adjusted to 5–6 with HCl (0.5 M, ca. 40 mL). The crude product was extracted with ethyl acetate and the organic phase was dried over sodium sulfate, filtered, concentrated under reduced pressure and purified by flash column chromatography (ethyl acetate–hexane, 3:2) to give HBED-CC-tris(*tert*-butyl ester) as a colorless solid (0.53 g, 0.76 mmol, 33.4%). *Note*: The overall product yield was 28%. Mp 43–45 °C. ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 7.00 (sept, 2 H, C*H*CHC-$(CH_2)_2CO_2$H and C*H*CHC-$(CH_2)_2CO_2t$-Bu), 6.76 (m, 4 H, CC*H*C-$(CH_2)_2CO_2$H, CC*H*C-$(CH_2)_2CO_2t$-Bu, CHC*H*C-$(CH_2)_2CO_2$H and CHC*H*C-$(CH_2)_2CO_2t$-Bu), 3.68 (d, 4 H, $^{Bn}CH_2$), 3.16 (d, 4 H, *t*-BuO_2CH_2N), 2.84 (t, $^3J_{H-H}$ = 7.6 Hz, 2 H, $CH_2CH_2CO_2$H), 2.78 (t, $^3J_{H-H}$ = 7.8 Hz, 2 H, CH_2CH_2-CO_2t-Bu), 2.66 (s br, 4 H, N(CH_2)$_2$N), 2.61 (t, $^3J_{H-H}$ = 7.6 Hz, 2 H, $CH_2CH_2CO_2$H), 2.46 (t, $^3J_{H-H}$ = 7.8 Hz, 2 H, $CH_2CH_2CO_2$t-Bu), 1.45 (d, 18 H, $NCH_2CO_2C(CH_3)_3$), 1.41 (s, 9 H, $CH_2CH_2CO_2C(CH_3)_3$). ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ = 176.93 (CO_2H), 172.72 ($CH_2CH_2CO_2t$-Bu), 170.30 ([NCH_2CO_2t-Bu]-ArCO_2H), 170.27 ([NCH_2CO_2t-Bu]-ArCO_2t-Bu), 155.94 (*C*OH), 155.71 (*C*OH), 131.52 (*C*-$(CH_2)_2CO_2$H), 130.87 (*C*-$(CH_2)_2CO_2t$-Bu), 129.44 (C*C*HC-$(CH_2)_2CO_2$H), 129.16 (C*C*HC-$(CH_2)_2CO_2t$-Bu), 129.11 (CH*C*HC-$(CH_2)_2CO_2$H), 129.08 (CH*C*HC-$(CH_2)_2CO_2t$-Bu), 121.71 (*C*CHC-$(CH_2)_2CO_2$H), 121.47 (*C*CHC-$(CH_2)_2CO_2t$-Bu), 116.63 (CH*C*HC-$(CH_2)_2CO_2$H), 116.46 (CH*C*HC-$(CH_2)_2CO_2t$-Bu), 82.38 ([$NCH_2CO_2CCH_3$]-ArCO_2H), 82.30 ([$NCH_2CO_2CCH_3$]-ArCO_2t-Bu), 80.49 (-$(CH_2)_2CO_2CCH_3$), 58.13 (^{Bn}*C*-ArCO_2H), 58.06 (^{Bn}*C*-ArCO_2t-*Bu*), 55.87 ([NCH_2CO_2]-ArCO_2H), 55.69 ([NCH_2CO_2]-ArCO_2t-Bu), 50.32 (NCH_2CH_2N), 37.59 ($CH_2CH_2CO_2t$-Bu), 35.88 ($CH_2CH_2CO_2$H), 30.39 ($CH_2CH_2CO_2t$-Bu), 30.01 ($CH_2CH_2CO_2$H), 28.20 (C(CH_3)). HRMS (ESI+, MeOH): *m*/*z* calcd for $C_{38}H_{57}N_2O_{10}$: 701.4008; found: 701.4027. HRMS (ESI–, MeOH): *m*/*z* calcd for $C_{38}H_{55}N_2O_{10}$: 699.3862; found: 699.3861. Anal. Calcd. (%) C: 65.12, H: 8.05, N: 4.00; found C: 65.35, H: 7.95, N: 3.91.

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