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Solid phase synthesis of peptide hydroxamic acids on poly(ethylene glycol)-based support

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A novel resin designed for solid-phase synthesis of peptide hydroxamic acids (PHA) combining the trityl linker with poly(ethylene glycol)-based support, ChemMatrix® type, is described. The synthesis of PHA can be performed according to a standard protocol, providing products in excellent purity and reasonable yields. Copyright © 2012 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: hydroxamic acid; ChemMatrix resin; modified peptide; solid phase

Introduction

Peptide hydroxamic acids (PHA) have been known for a long time as strong metal chelators [1,2] and inhibitors of metalloenzymes [3]. Some of them have been introduced into clinical trials as potential drugs for cancer, arthritis and other diseases [4].

Their common inhibitory effect is based on the complexing of the metal ion in the enzyme active center by the hydroxamate group [5]. These interactions result in deactivation of the enzyme. To improve the effectiveness and specificity of these ligands as inhibitors, numerous hydroxamic acids have been synthesized and tested. [3,6–10]

Recently, there has been an increasing interest in formation of supramolecular structures by various derivatives of hydroxamic acids, especially aminohydroxamic acids. The literature data illustrate the examples of metallacrowns – the structures formed by 4 hydroxamates and 5 copper (II) ions [11]. These complexes are formed by α, β and γ-aminohydroxamic acids [12]. Hydroxamate derivatives of α-acetyl histidine can be also included into this class of ligands [13].

The PHA can be obtained by several methods, including solid phase synthesis. The literature describes various strategies allowing the synthesis of PHA on solid support [14–18]. However, in most cases, the solid phase synthesis of hydroxamic acids is limited to compounds with relatively low molecular mass. In this work, we propose a new support for solid phase synthesis of PHA that is based on derivatized trityl ChemMatrix® resin. A series of PHA containing His and β-Asp amino acid residues at C-termini that potentially can form the metallacrown type supramolecular structures were prepared. The amino acid sequences were based on amphiphilic peptides applied by Mutter in designing TASP molecules [19]. The results obtained on this support were compared with those obtained using commercially available hydroxylamine Wang resin demonstrating that a new resin allows a synthesis of difficult sequences with hydroxamic acid moiety.

Material and Methods

Reagents

All solvents and reagents were used as supplied. The Fmoc amino acids derivatives (Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OBu^t)-OH and Fmoc-His(Trt)-OH) and N-hydroxyphtalimide (PHT-NOH) were purchased from Novabiochem (Merck, Darmstadt, Germany). The *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)-methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide (TBTU) and trifluoroacetic acid (TFA) were obtained from IrisBiotech (Marktredwitz, Germany). Diisopropylethylamine (DIEA), dimethylformamide (DMF), hydrazine and trityl ChemMatrix® resin were obtained from Sigma-Aldrich (St. Louis, MO, USA). The hydroxylamine Wang resin and piperidine were purchased from Merck (Darmstadt, Germany). Triisopropylsilane (TIS) was purchased from Fluka (Sigma-Aldrich). Solvents for NMR experiments were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Other chemicals were obtained from POCH (Gliwice, Poland).

Synthesis

General peptides synthesis

Solid phase peptide synthesis (SPPS) of model peptides (peptides 1–5) were preformed manually in polypropylene syringe reactors (Intavis AG) equipped with polyethylene filters, according to standard Fmoc procedure [20]. The Fmoc – protecting groups were removed using 25% piperidine solution in DMF. The coupling reactions were performed in DMF using TBTU (3 eq) in presence of DIEA (3 eq). The reaction was monitored by Kaiser test [21]. Peptide 1 was synthesized on commercially available hydroxamine Wang resin. The other compounds were prepared on hydroxamine type ChemMatrix® resin prepared in our laboratory according to procedure given in the succeeding text. The

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peptides were cleaved from the resin using a solution of TFA/H_2O/TIS (95 : 2.5 : 2.5) at room temperatures for 2 h. The peptides 1 and 3 were precipitated with cold diethyl ether (Et_2O). The peptides 3, 4 and 5 were isolated after evaporation of TFA in nitrogen stream. The yields of crude peptides estimated on a dry weight basis (assuming that peptides were isolated in the trifluoroacetates form) were typically 60–70%.

The sequences of synthesized peptides are shown in Figure 1

Analytical data of obtained peptides:

[Peptide 1]

ESI-MS: $[M+2H]^{2+}=860.5226$ (calcd $m/z=860.5276$ for $C_{78}H_{140}N_{22}O_{21}$); ESI–MS/MS ($m/z$ value for the most abundant peaks): parent ion $[M+2H]^{2+}$: $[M\text{-}NHOH+H]^{2+}=844.0166$ (calcd $m/z=844.0169$ for $C_{78}H_{137}N_{21}O_{20}$); $b_{14}^{2+}=775.4875$ (calcd $m/z=775.4874$ for $C_{72}H_{130}N_{18}O_{19}$); $b_{13}^{2+}=718.9456$ (calcd $m/z=718.9454$ for $C_{66}H_{119}N_{17}O_{18}$); $b_{12}^{+}=1308.7887$ (calcd $m/z=1308.7886$ for $C_{60}H_{106}N_{15}O_{17}$); $b_{10}^{+}=1124.665$ (calcd $m/z=1124.6674$ for $C_{51}H_{90}N_{13}O_{15}$); $b_{9}^{+}=1053.6302$ (calcd $m/z=1053.6303$ for $C_{48}H_{85}N_{12}O_{14}$); $b_{6}^{+}=683.4089$ (calcd $m/z=683.4087$ for $C_{31}H_{55}N_8O_9$); $b_{5}^{+}=612.3717$ (calcd $m/z=612.3715$ for $C_{28}H_{50}N_7O_8$).

R_{t1} 28.2 min, R_{t2} 26.0 min, Yield of purified peptide 36%

[Peptide 2]

ESI-MS: $[M+2H]^{2+}=896.0466$ (calcd $m/z=896.0462$ for $C_{81}H_{145}N_{23}O_{22}$). ESI–MS/MS: ($m/z$ value for the most abundant peaks): parent ion $[M+2H]^{2+}$: $[M\text{-}NHOH+H]^{2+}=879.5345$ (calcd $m/z=879.5354$ for $C_{81}H_{142}N_{22}O_{21}$); $b_{15}^{2+}=811.0062$ (calcd $m/z=811.0060$ for $C_{75}H_{135}N_{19}O_{20}$); $b_{14}^{2+}=775.4875$ (calcd $m/z=775.4874$ for $C_{72}H_{130}N_{18}O_{10}$); $b_{13}^{2+}=718.9458$ (calcd $m/z=718.9454$ for $C_{66}H_{119}N_{17}O_{18}$); $b_{12}^{2+}=654.8971$ (calcd $m/z=654.8979$ for $C_{60}H_{107}N_{15}O_{17}$); $b_{10}^{+}=1124.6627$ (calcd $m/z=1124.6674$ for $C_{51}H_{90}N_{13}O_{15}$); $b_{9}^{+}=1053.6282$ (calcd $m/z=1053.6303$ for $C_{48}H_{85}N_{12}O_{14}$); $b_{8}^{+}=924.5859$ (calcd $m/z=924.5877$ for $C_{43}H_{78}N_{11}O_{11}$); $b_{7}^{+}=796.4931$ (calcd $m/z=796.4927$ for $C_{37}H_{66}N_9O_{10}$); $b_{6}^{+}=683.4083$ (calcd $m/z=683.4087$ for $C_{31}H_{55}N_8O_9$); $b_{5}^{+}=612.3701$ (calcd $m/z=612.3715$ for $C_{28}H_{50}N_7O_8$); $y_{5}^{+}=554.3389$ (calcd $m/z=554.3409$ for $C_{24}H_{44}N_9O_6$); $y_{7}^{+}=738.4631$ (calcd $m/z=738.4621$ for $C_{33}H_{60}N_{11}O_8$), $y_{13}^{+}=1436.8648$ (calcd $m/z=1436.8584$ for $C_{64}H_{114}N_{19}O_{18}$); $y_{14}^{+}=1549.9426$ (calcd $m/z=1549.9424$ for $C_{70}H_{123}N_{20}O_{19}$); $y_{15}^{+}=1620.9836$ (calcd $m/z=1620.9796$ for $C_{73}H_{130}N_{21}O_{20}$).

R_{t1} 30.4 min, R_{t2} 25.6 min, Yield of purified peptide 40%

[Peptide 3]

ESI-MS: $[M+2H]^{2+}=870.5404$ (calcd 870.5407 for $C_{78}H_{144}N_{22}O_{22}$); ESI-MS/MS: ($m/z$ value for the most abundant peaks): parent ion $[M+2H]^{2+}$: $[M\text{-}NHOH+H]^{2+}=854.0307$ (calcd $m/z=854.0300$ for $C_{78}H_{141}N_{21}O_{21}$), $b_{14}^{+}=775.4871$ (calcd $m/z=775.4874$ for $C_{72}H_{130}N_{18}O_{19}$), $b_{13}^{2+}=718.9455$ (calcd 718.9454 for $C_{68}H_{129}N_{19}O_{14}$), $b_{12}^{+}=1308.7889$ (calcd $m/z=1308.7886$ for $C_{62}H_{116}N_{17}O_{13}$), $b_{11}^{+}=1237.7479$ (calcd $m/z=1237.7514$ for $C_{57}H_{101}N_{14}O_{16}$), $b_{10}^{+}=1124.667$ (calcd $m/z=1124.6674$ for $C_{51}H_{90}N_{13}O_{15}$), $b_{9}^{+}=1053.6303$ (calcd $m/z=1053.6303$ for $C_{48}H_{85}N_{12}O_{14}$). $b_{8}^{+}=924.5879$. (calcd 924.5878 for $C_{43}H_{78}N_{11}O_{11}$), $b_{5}^{+}=612.3697$ (calcd 612.3715 for $C_{28}H_{50}N_7O_8$).

R_{t1} 28.8 min, R_{t2} 26.4 min, Yield of purified peptide 24%

[Peptide 4]

ESI-MS: $[M+2H]^{2+}=869.5934$ (calcd $m/z=869.5931$ for $C_{80}H_{154}N_{24}O_{18}$); ESI-MS/MS: ($m/z$ value for the most abundant peaks): parent ion $[M+2H]^{2+}$: $[M\text{-}NHOH+H]^{2+}=853.0821$ (calcd $m/z=853.0824$ for $C_{80}H_{151}N_{23}O_{17}$); $b_{14}^{2+}=774.5395$ (calcd $m/z=774.5398$ for $C_{74}H_{140}N_{20}O_{15}$); $b_{13}^{+}=1434.9881$ (calcd $m/z=1434.9883$ for $C_{68}H_{128}N_{19}O_{14}$); $b_{13}^{2+}=717.9978$ (calcd $m/z=717.9978$ for $C_{68}H_{129}N_{19}O_{14}$); $b_{12}^{+}=1306.8956$ (calcd $m/z=1306.8933$ for $C_{62}H_{116}N_{17}O_{13}$); $b_{12}^{2+}=653.9504$ (calcd $m/z=653.9503$ for $C_{62}H_{117}N_{17}O_{13}$); $b_{11}^{+}=1235.8562$ (calcd $m/z=1235.8562$ for $C_{59}H_{111}N_{16}O_{12}$); $b_{9}^{+}=1051.7343$ (calcd $m/z=1051.7350$ for $C_{50}H_{95}N_{14}O_{10}$); $b_{8}^{+}=923.6404$ (calcd $m/z=923.6400$ for $C_{44}H_{83}N_{12}O_9$); $b_{6}^{+}=682.4609$ (calcd $m/z=682.4610$ for $C_{32}H_{60}N_9O_7$); $b_{5}^{+}=611.4245$ (theoretical $m/z=611.4239$ calculated for $C_{29}H_{55}N_8O_6$); $y_{4}^{+}=503.3311$ (calculated $m/z=503.3300$ for $C_{21}H_{43}N_8O_6$), $y_{7}^{+}=815.5455$ (calcd $m/z=815.5461$ for $C_{36}H_{70}N_{12}O_9$), $y_{8}^{+}=943.6407$ (calcd $m/z=943.6411$ for $C_{42}H_{83}N_{14}O_{10}$), $y_{9}^{+}=1056.7245$ (calcd $m/z=1056.7252$ for $C_{48}H_{94}N_{15}O_{11}$), $y_{10}^{+}=1127.7610$ (calcd $m/z=1127.7623$ for $C_{51}H_{89}N_{16}O_{12}$).

R_{t1} 25.4 min, R_{t2} 22.5 min, Yield of purified peptide 32%

[Peptide 5]

ESI-MS: $[M+2H]^{2+}=872.4363$ (calcd $m/z=872.4360$ for $C_{74}H_{122}N_{18}O_{30}$); ESI-MS/MS: ($m/z$ value for the most abundant peaks): parent ion $[M+2H]^{2+}$: $b_{13}^{+}=1440.6798$ (calcd $m/z=1440.6740$ for $C_{62}H_{98}N_{13}O_{26}$), $b_{12}^{+}=1311.6315$ (calcd $m/z=1311.6315$ for $C_{57}H_{91}N_{12}O_{23}$), $b_{11}^{+}=1240.5965$ (calcd $m/z=1240.5943$ for $C_{54}H_{86}N_{11}O_{22}$), $b_{10}^{+}=1127.5115$ (calcd $m/z=1127.5103$ for $C_{48}H_{75}N_{10}O_{21}$), $b_{9}^{+}=1056.4733$ (calcd $m/z=1056.4732$ for C45H70N9O20), $b_{8}^{+}=927.4310$ (calcd $m/z=927.4306$ for C40H63N8O17),

Ac-Lys-Ala-Leu-Glu-Lys-Ala-Leu-Lys-Glu-Ala-Leu-Ala-Lys-Leu-His-NHOH
Peptide 1

Ac-Lys-Ala-Leu-Glu-Lys-Ala-Leu-Lys-Glu-Ala-Leu-Ala-Lys-Leu-βAla-His-NHOH
Peptide 2

Ac-Lys-Ala-Leu-Glu-Lys-Ala-Leu-Lys-Glu-Ala-Leu-Ala-Lys-Leu-Xxx
Peptide 3

Ac-Lys-Ala-Leu-Lys-Lys-Ala-Leu-Lys-Lys-Ala-Leu-Ala-Lys-Leu-Xxx
Peptide 4

Ac-Glu-Ala-Leu-Glu-Glu-Ala-Leu-Glu-Glu-Ala-Leu-Ala-Glu-Leu-Xxx
Peptide 5

Xxx :

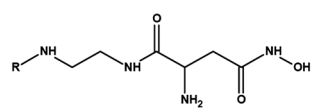

Figure 1. The sequences of synthesized peptide hydroxamic acids.

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$b_7^+ = 798.3883$ (calcd $m/z = 798.3880$ for $C_{35}H_{56}N_7O_{14}$), $b_6^+ = 685.3042$ (calcd $m/z = 685.3039$ for $C_{29}H_{45}N_6O_{13}$), $b_5^+ = 614.2673$ (calcd $m/z = 614.2668$ for $C_{26}H_{40}N_5O_{12}$), $b_3^+ = 356.1816$ (calcd $m/z = 356.1816$ for $C_{16}H_{26}N_3O_6$).

R_{t1} 36.6 min, R_{t2} 30.2 min, Yield of purified peptide 31%

Synthesis of Asp Derivative – Boc-Asp-EDA-Fmoc

The Boc-Asp-EDA-Fmoc was synthesized according to scheme shown in Figure 2.

Z-EDA-Boc

Z-EDA-Boc was obtained from Boc-EDA-H. Synthesis of Boc-EDA-H has been previously described in the literature by Reschel *et al.* [22]. Boc-EDA-H (0.027 mol, 4.26 g) was dissolved in the mixture of 46 ml 1 M aqueous sodium carbonate and 125 ml acetone. To a stirred solution, benzyloxycarbonyl chloride (0.027 mol, 3.8 ml) was added dropewise. The reaction was allowed to proceed for 24 h. The partially precipitated product was filtered. The filtrate was extracted with ethyl acetate. The solvent was removed *in vacuo*, and the next batch of product was isolated by crystallization from the mixture of water and acetone.

Yield 51%

Melting point: 168–170.1 °C (lit. 169–172 °C)

^{1}H NMR($CDCl_3$) 500 MHz: $\delta = 1.43$ (9H, s); $\delta = 3.26$(4H, m); $\delta = 4.79$ (0.87, br-s); $\delta = 5.08$ (2.76H, s); $\delta = 7.31$(5H, m).

*Z-EDA*HCl*

In 50 ml THF, 0.013 mol (3.82 g) of Z-EDA-Boc was dissolved. The dry HCl gas was bubbled through solution for 1 h. Then the product was isolated by filtration, washed with diethyl ether and dried *in vacuo*.

Yield 87%

Melting point 122.1–124.8 °C (lit. 123–124 °C)

^{1}H NMR (D_2O) 500 Hz $\delta = 3.18$ (2H t, $J^3 = 5.88$ Hz), $\delta = 3.5$ (2H t, $J^3 = 5.69$ Hz), $\delta = 5.19$ (2H s), $\delta = 7.49$ (5H m).

Boc-Asp(OBzl)-ONSu

Synthesis of N-hydroxysuccinimide ester was performed according to Anderson *et al.* [23] using DCC as a coupling reagent. Boc-Asp-OH (0.03 mol, 10 g) and N-hydroxysuccinimide (0.03 mol, 3.57 g) were dissolved in dioxane (60 ml). The solution was cooled to 5 °C, and then the DCC (0.03 mol, 4.76 ml) was added. The solution was stirred at 0 °C for 24 h. Next, the solvent was removed *in vacuo*, and the crude product was precipitated by addition of pentane. The product was used for further synthesis without purification.

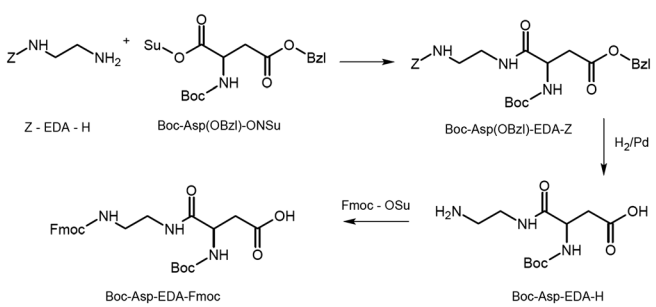

Figure 2. Scheme of synthesis of Boc-Asp-EDA-Fmoc.

Yield 98%

Melting point 95–97 °C (lit. [24] 103–104 °C)

^{1}H NMR ($CDCl_3$) 500 MHz: $\delta = 1.43$ (9H s); $\delta = 2.81$ (4H s); $\delta = 2.96$ (1H dd, $J^3 = 4.47$ Hz, $J^2 = 17.48$ Hz); $\delta = 3.13$ (1H dd, $J^3 = 4.67$ Hz, $J^2 = 17.39$ Hz); $\delta = 5.00$ (1H m); $\delta = 5.18$ (2H m); $\delta = 5.58$ (1H d, $J^3 = 9.0$ Hz); $\delta = 7.34$ (5H m).

Boc-Asp(OBzl)EDA-Z

The synthetic procedure was similar to that described by Anderson [25]. To the solution of Boc-Asp(OBzl)-ONSu (0.005 mol, 2.1 g) in THF (35 ml) and DIEA (0.01 mol, 1,73 ml), the Z-EDA-H*HCl (0.005 mol, 1.15 g) was added. After 1 h at room temperature, the product was precipitated from the solution by adding 100 ml of water. After filtration, the precipitate was washed with 10% sodium bicarbonate, water, 1M hydrochloric acid, water and finally dried *in vacuo*.

Yield: 71%

^{1}H NMR ($CDCl_3$) 500 Hz : $\delta = 1.42$ (9H,s), $\delta = 2.7$ (1H dd, $J^3 = 5.96$ Hz, $J^2 = 17.1$ Hz), $\delta = 3.04$ (1H dd, $J^3 = 4.6$ Hz, $J^2 = 17.2$), $\delta = 3.34$ (4H m), $\delta = 4.45$ (1H s), $\delta = 5.08$ (4H m), $\delta = 5,21$ (1H s), $\delta = 5.52$ (1H s), $\delta = 6.71$ (1H s), $\delta = 7.32$ (10H m).

Boc-Asp-EDA-H

The protecting groups (Z and Bzl) were removed by hydrogenation in the presence of 10% Pd on C in the mixture of isopropanol/methanol (5 : 5 v : v) under atmospheric pressure. The substrate Boc-Asp(OBzl)-EDA-Z (0.012 mol, 6 g) was dissolved at 40 °C in the 80 ml of solvent. After 3 h of hydrogenation, the catalyst was filtered off, and the solvent was removed *in vacuo*. The product was used for further synthesis without purification.

Yield 85%

ESI-MS: $[M + H]^+ = 276.1625$ (calcd 2716.1554 for $C_{11}H_{21}N_3O_5$)

Boc-Asp-EDA-Fmoc

The protection of amino group with 9-fluorenylmethyloxycarbonyl was performed according modified procedure given by Paquet [26]. Boc-Asp-EDA-H (0.008 mol, 2.2 g) and DIEA (0.008 mol, 1.4 ml) were dissolved in mixture of water (10 ml) and acetone (34 ml). The mixture was heated until substrate dissolved. After cooling the solution to room temperature Fmoc-OSu (0.008 mol, 2.7 g) was added. The reaction mixture was stirred overnight, and then the acetone was removed *in vacuo*. The product was precipitated by addition of 1M solution of $KHSO_4$ to pH = 2, filtered off, washed with water and dried.

Yield 92%

Melting point 153–155 °C

ESI-MS: $[M + H]^+ = 498.2170$ (calcd 498.2235 for $C_{26}H_{32}N_3O_7$)

^{1}H NMR (DMSO-d_6) 500M Hz $\delta = 1.37$ (9H s), $\delta = 2.44$ (1H dd $J^2 = 16.87$, $J^3 = 8.04$ Hz), $\delta = 2.62$ (1,41H dd $J^2 = 16.87$ $J^3 = 5.09$ Hz), $\delta = 3.04$ (2H m), $\delta = 3.1$ (2H m), $\delta = 4.21$ (2H m), $\delta = 4.29$ (2H m), $\delta = 6.93$ (1H d $J^3 = 8.03$ Hz), $\delta = 7.24$ (1H t $J^3 = 5.32$ Hz), $\delta = 7.33$ (2H m), $\delta = 7.41$ (2H m), $\delta = 7.67$ (2H m), $\delta = 7.88$ (2H m), $\delta = 12.06$ (0.66H br-s).

Derivatization of Trityl ChemMatrix® Resin

Derivatization of ChemMatrix® support, shown in Figure 3, was performed according to Bauer's procedure [27] developed previously for a cross-linked polystyrene Wang resin.

A sample of ChemMatrix® resin (100 mg) was suspended in DCM (2 ml) for about 30 min. Then the solution of 2% $SOCl_2$ in DCM was added (3 ml). The reaction was allowed to proceed for

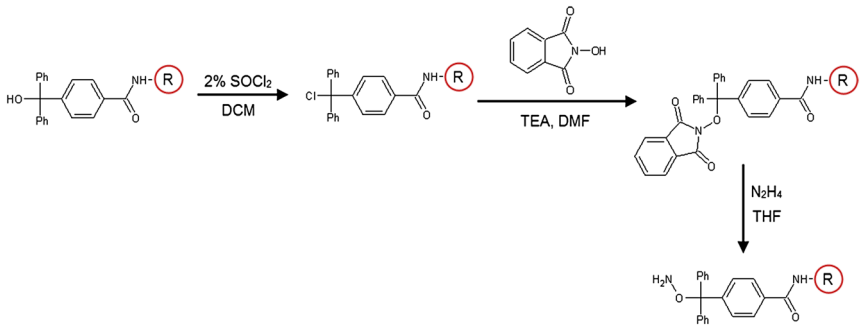

Figure 3. Scheme of hydroxylamine ChemMatrix® resin preparation.

48 h with exchange of thionyl chloride solution after 24 h. Then the resin was washed with DCM (7 × 1 min × 2 ml), N-methylmorpholine (2% in DCM; 3 × 1 min × 2 ml), DCM (5 × 1 min × 2 ml), THF, H_2O, DCM and DMF. The solution of N-hydroxyphtalimide (5 eq), TEA (5 eq) in 3 ml DMF was added to the resin. The reaction was allowed to proceed for 48 h, with exchange of reagents solution after 24 h. The resin was collected by filtration and washed successively (3 × 1 min × 2 ml) with DMF, water, THF, MeOH, diethyl ether, and dried *in vacuo*. Then the resin was suspended in THF (about 3 ml, 30 min) and after removing THF, the mixture of hydrazine hydrate and THF (1 : 10 v : v) was added. After 24 h, the THF–hydrazine mixture was exchanged, and the reaction was continued for the next 24 h. Finally, the resin was separated, washed successively with THF, DMF, 5 M ammonium hydroxide solution, water, THF, MeOH, diethyl ether, and dried *in vacuo*.

General Methods

Purification

All crude products were analyzed by the analytical HPLC using Thermo separation HPLC system with UV detection (210 nm) on a Vydac Protein RP C18 column (250 × 4.6 mm, 5 μm) (Grace, Deerfield, IL, USA), with gradient elution of 0–80% B in A (A = 0.1% TFA in water; B = 0.1% TFA in acetonitrile/H_2O, 4 : 1) over 45 min (flow rate 1 ml/ml, rt). For all compounds, additional HPLC analysis was performed on Varian Microsorb-MV 100–5 CN column (4.6 mm × 250 mm; Varian, Palo Alto, CA, USA) in this same gradient system. Retention times are given as R_{t1} and R_{t2}, respectively. Preparative reversed-phase HPLC was performed on Tosoh TSKgel with a ODS-120T column (21.5 mm × 300 mm) (Tosoh, Tokyo, Japan) using the same solvent system, gradient 0.5%/min and flow rate 7 ml/min.

Mass spectrometry

The MS experiments of peptides were performed on an FT-ion cyclotron resonance (ICR) MS Apex-Qe Ultra 7T instrument (Bruker Daltonic, Germany) equipped with an ESI source. The spectra of products were recorded in the solution of acetonitrile : water : formic acid (50% : 50% : 0.1%). Argon was used as a collision gas. The instrument was operated in positive ion mode and calibrated with Tunemix™ (Bruker Daltonic). The instrumental parameters were the following: temperatures of drying gas was 200°C, the potential between the spray needle, and the orifice was set to 4.5 kV, source accumulation 0.5 s, ion accumulation time 0.5 s. For each product, the collision energy was optimized for the best fragmentation. In MS/MS mode, the precursor ions were selected in the quadrupole collision cell and subsequently fragmented in the hexapole collision cell.

The spectra of low molecular mass intermediate compounds were recorded on Bruker micrOTOF-Q mass spectrometer equipped with Apollo II electrospray ionization source. The instrumental parameters were the following: compound was dissolved in the solution acetonitrile : water : formic acid (50% : 50% : 0,1%), scan range *m/z* 300–1600, dry gas–nitrogen (3 L/min), temperature 200 °C, and ion energy 5 eV.

Nuclear magnetic resonance

The 1D and 2D spectra of intermediate products were recorded on Bruker Avance 500 MHz spectrometer. The experiments were performed at 25 °C. The solvents were selected appropriately to each compound (see synthesis of Asp derivative). The concentration of each compound was approx. 10 mg/ml.

Peptide 3 (2 mg) was dissolved in 99% D_2O (650μL). The pH value was in the range of 4.3–4.8. All measurements were conducted at 25°C. The 1D and 2D NMR experiments were recorded at 11.7 T on Varian Unity + 500 spectrometer. The 2D dimensional spectra (homonuclear and heteronuclear) were processed by NMRPipe [28] and analyzed with Sparky [29] programs. The assignment of non-exchangeable ^{1}H and ^{13}C resonances for all amino acids spin systems was performed by application of a standard procedure [30] based on 2D homonuclear TOCSY (mixing times 10 and 90 ms) and ROESY (mixing time 200 and 500 ms) experiments supplemented by 2D heteronuclear ^{1}H–^{13}C HSQC spectrum, recorded on natural abundances of ^{13}C nuclei.

Melting points

Melting points (uncorrected) were measured with a Boethius PHMK (VEB Analityk, Dresden, Germany) apparatus.

Result and Discussion

Although the data presented in literature gives several examples of synthesis of peptide hydroxamic acids, the majority of these papers describe relatively simple compounds with low molecular weight. In our research, we tested commercially available resin designed for the solid phase synthesis of hydroxamic acids. The attempts on synthesis of simple hydroxamic acids (not presented) or short peptides were successful; however, longer sequences (more than six amino acid residues) were not obtained with acceptable purity. To solve this problem, we decided to implement the known synthetic approach based on derivatized trityl linker [27] on ChemMatrix® resin. The derivatized support was prepared according to the procedure given

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in the experimental part, using commercially available trityl alcohol ChemMartix® resin and N-hydroxyphtalimide as a hydroxylamine source.

ChemMatrix® resin has been previously reported as extremely efficient support in solid phase synthesis of long or difficult sequences [31]. We presented a modified method of synthesis of polypeptides containing aminohydroxamic acid moieties in C-terminal part. This method allows to obtain relatively long polypeptides with excellent purities. To compare the resin prepared according to our procedure with commercially available hydroxylamine Wang support, we synthesized Peptide 1 simultaneously on these two resins. During the synthesis, after attaching the 6th and 10th amino acid residue and after completing the synthesis the resulting crude products were characterized using HPLC and ESI-MS methods (Figure 4). The chromatographic monitoring of solid phase synthesis revealed that short sequences may be successfully synthesized on both commercial polystyrene based resin as well as the ChemMatrix® support derivatized according to our protocol. However, for longer sequences, especially for peptides composed of 15 amino acid residues, the advantage of ChemMatrix support is evident.

The products obtained on hydroxylamine ChemMatrix® resin have much higher purity (Figure 4) than those obtained on hydroxylamine Wang resin. This observation is in agreement with general knowledge that ChemMatrix® resin overcomes the challenges of synthesizing difficult sequences.

The ChemMatrix® based hydroxamine resin was further tested in a synthesis of a series of peptide containing β-aminohydroxamic acid moiety in C-terminal part. Also, model peptides with hydroxamic residue attached directly to N-terminal histidine were obtained. On the basis of previous reports, these peptides are likely to form supramolecular, tetrameric associates connected by the metallacrown structure. [12]

The histidine containing systems were obtained by standard Fmoc strategy. To synthesize peptides substituted in C-terminal part with β-aminohydroxamic residue, the aspartic acid derivative (Boc-Asp-EDA-Fmoc) was designed. The Boc-Asp-EDA-Fmoc was obtained separately in solution according to Figure 3. Then, the obtained derivative was attached to the hydroxylamine derivatized ChemMatrix® resin, and the synthesis was performed in the standard way.

Obtained crude peptides were characterized by HPLC and ESI-MS to check their purity. After purification by preparative HPLC, synthetic peptides were analyzed by analytical HPLC in two systems, HR-MS to confirm the elemental composition and MS/MS to verify the sequence. Additionally, one representative example, peptide 3, was investigated by NMR technique. The 1D ^{1}H NMR spectrum of the peptide 3 is presented in Figure 5. The signals are heavily overlapping because of multiple amino acid repeats present within the sequence. The integration analysis of the spectral ranges typical for resonances characteristic for selected amino acids allowed to confirm the amino acids composition. It was also possible to assign unambiguously the amino acid residues with the combination of 2D ^{1}H–^{1}H TOCSY and ^{1}H–^{1}H ROESY experiments. All 15 from 15 expected Hα/Cα correlations have been found together with acetyl as well as diaminoethyl moieties (Figure 6). The analysis of Cα chemical shift values indicates that peptide 3 is not structured in solution.

The HR-MS as well as fragmentation spectra of all peptides and NMR data for peptide 3 unambiguously confirmed structures of obtained compounds.

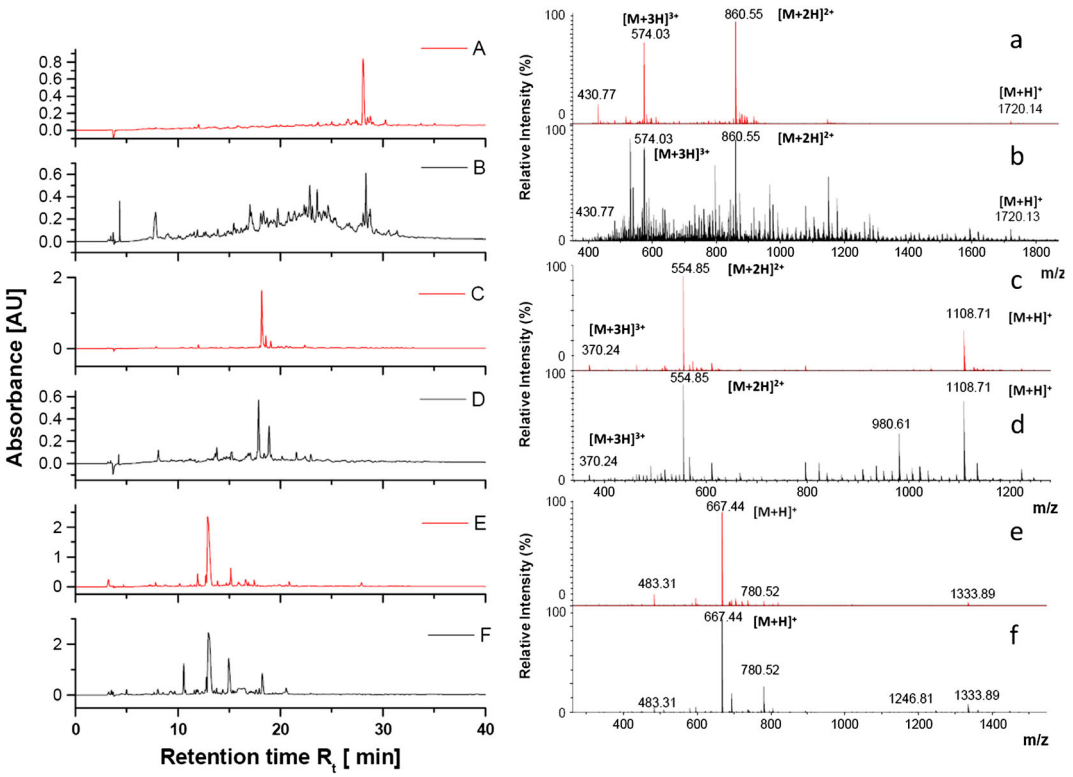

Figure 4. The comparison of synthesis of peptide 1 performed on new modified hydroxylamine type ChemMatrix® resin (panels A,C,E and a,c,e) and commercially available polystyrene Wang resin (B,D,F and b,d,f). Each MS spectrum (right) corresponds to the chromatogram (left) of the same sample. A,a, B,b – crude products containing 15 amino acid residues; C,c, D,d – fragment [6–15] of Peptide 1; E,e, F,f – fragment [10–15] of Peptide 1.

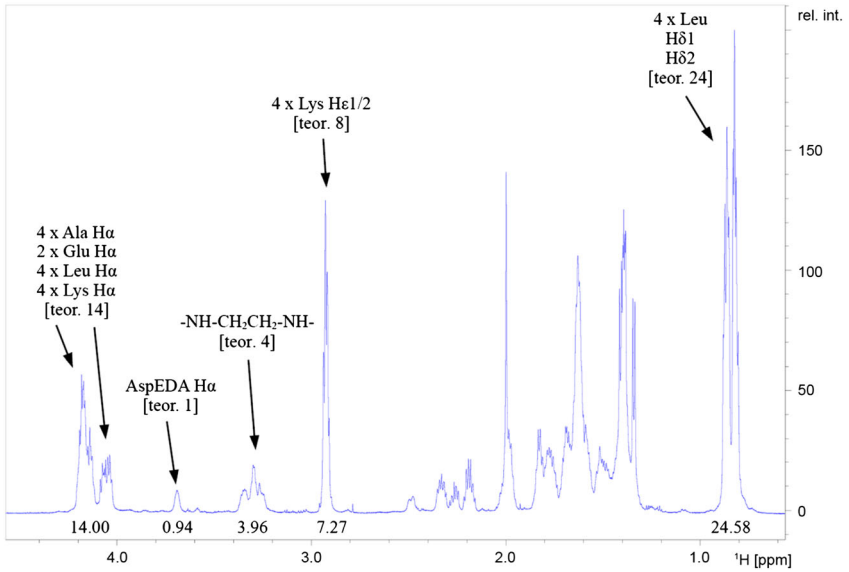

Figure 5. The 1D ^{1}H NMR spectrum of peptide 3 in D_2O recorded at 25 °C. Selected spectral fragments corresponding to signals from certain protons within the peptide are assigned and integrated.

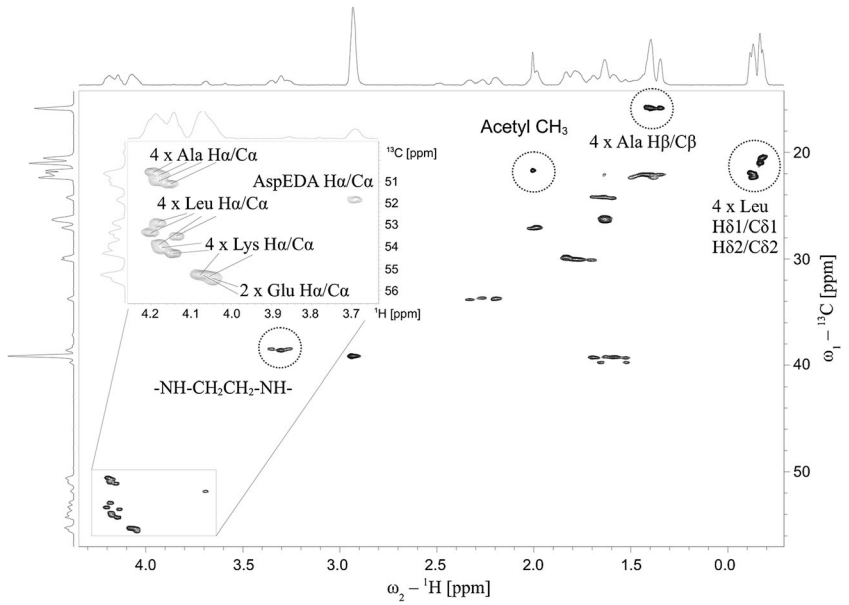

Figure 6. The 2D ^{1}H-^{13}C HSQC spectrum of peptide 3 in D_2O. Diagnostic $^{1}H/^{13}C$ correlations for acetyl, diaminoethyl, leucine, and alanine side chain methyl moieties are marked by dotted circles. The assignments of 15 well separated from 15 expected Hα/Cα correlations is presented.

Conclusion

In this paper, a new variant of ChemMartrix® resin derivatized to allow the synthesis of C-terminal hydroxamic acid peptides was described. The comparison of our resin with commercially available support based on cross-linked polystyrene shows superiority of derivatized ChemMatrix® resin.

A series of peptides with the hydroxamate moiety in the C-terminal part was obtained. The identity and homogeneity of these compounds have been proved using various analytical methods including HR-MS, MS/MS, HPLC, and NMR. Obtained compounds will be tested for their ability to form supramolecular structures of metallacrown type.

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