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Synthesis of 4-mercapto-L-lysine derivatives: Potential building blocks for sequential native chemical ligation

Kalyan Kumar Pasunooti [a,†], Renliang Yang [b,†], Seenuvasan Vedachalam [a], Bala Kishan Gorityala [a], Chuan-Fa Liu [b,*], Xue-Wei Liu [a,*]

[a] Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, Singapore 637371, Singapore
[b] Chemical Biology and Biotechnology Division, School of Biological Sciences, Nanyang Technological University, Singapore 637551, Singapore

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ABSTRACT

A general and diastereoselective synthesis of (2*S*, 4*S*)-4-mercapto-L-lysine derivative was described. The key features of this synthesis include Zn-mediated diastereoselective Reformatsky reaction and selective reduction of methyl ester with sodium borohydride. Introduction of thiol functional group on lysine side chain proved to be appropriate for dual native chemical ligation. This methodology allows to develop various 4-substituted L-lysine derivatives.

Cysteine-mediated native chemical ligation become the most attractive methodology for the preparation of novel peptides and protein domains.[1] Since its development it has become a powerful tool for the chemical synthesis of linear peptides.[2] This technique relies on the combination of a N-terminal cysteine residue with a C-terminal thioester to form a peptide bond.[3–5] Unfortunately, very few natural thiol-containing amino acids are available for peptide ligation to form new type peptides. For the past few years various chemical ligation methodologies have been developed. Wong and co-workers developed cysteine-free ligation by incorporating a thiol-containing sugar auxiliary on N-terminal serine, which allows direct access to glycopeptides.[6] In this Letter, we described the synthesis of 4-mercapto-L-lysine which serves as a pivotal building block for the assembly of branched peptides by native chemical ligation.

We chose 4-mercapto-L-lysine derivative as a mediator for dual native chemical ligation for the following reasons. (1) thio group lies between the α-amino group and the side chain amino group of lysine, which allows double native chemical ligation and furnishes branched peptides, as shown in Figure 1. Likely, this new branched structure endows the peptides or proteins with new property and capacity. (2) L-lysine is an essential amino acid and a necessary building block of proteins in the body. The substituted L-lysine derivatives, such as (2*S*,4*R*)-4-fluoro-L-lysine **2** is a bioactive molecule which enhances the biological activity relative to the parent molecule.[7] The hydroxyl analogue **3a** and **3b** are also useful precursors in many biological active components (Fig. 2).[8] Synthesis of these functionalized L-lysine derivatives is a challenging task due to the multiple functionality of L-lysine. In this work, we developed a new methodology to introduce a thio group onto the 4-position of L-lysine side chain by exploiting Reformatsky reaction as a key step.

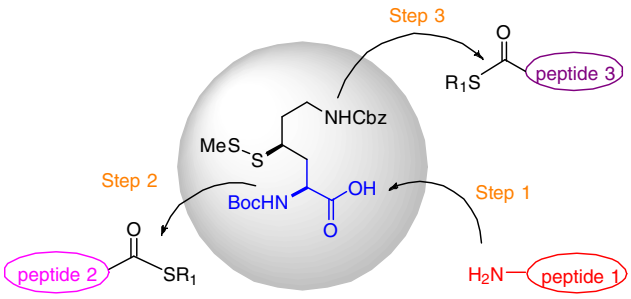

Figure 1. 4-Mercapto-L-lysine mediated sequential native chemical ligation that provides branched peptides.

We envisioned that the chirally pure target molecule, (2*S*,4*S*)-4-mercapto-L-lysine could be assembled from commercially available L-aspartic acid **4.** The side chain homologation, stereoselective introduction of thiol and amino groups are the key steps in our strategy. As shown in Scheme 1, the conversion of L-aspartic acid

* Corresponding authors. Tel.: +65 6316 8901; fax: +65 6791 1961 (X.-W.L.).
E-mail addresses: CFLiu@ntu.edu.sg (C.-F. Liu), xuewei@ntu.edu.sg (X.-W. Liu).
† K. K. Pasunooti and R. L. Yang contributed equally to this paper.

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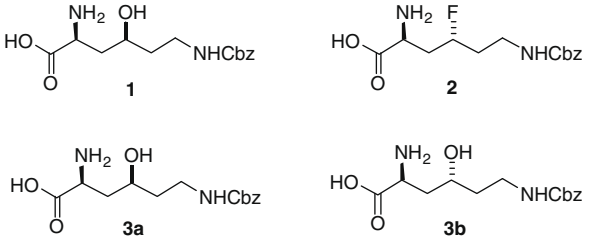


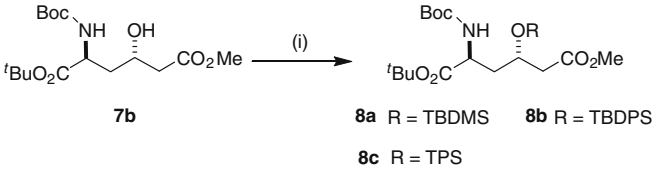
Figure 2. Some of 4-substituted L-lysine derivatives.

4 to α-*tert*-butyl-(*S*)-*N*-*tert*-butoxycarbonyl aspartate **5** in four steps with good yields.[9] Further, the protected aspartate **5** was transformed in to the corresponding thioester through DCC coupling at room temperature (yield 95%). The resulting thioester was reduced with the aid of triethylsilane—10% Pd/C to provide (*S*)-aspartate semi-aldehyde **6** in 85% yield.[10]

As shown in Scheme 2, the homologation of the side chain was carried out via diastereoselective Reformatsky reaction between (*S*)-aspartate semi-aldehyde **6** and methyl bromoacetate.[11] The resulting Reformatsky product has the same carbon skeleton as in the target molecule, which allows easy access to a series of 4-substituted lysine analogues by nucleophilic substitution of hydroxyl group. Optimization of reaction conditions identified, zinc and trimethylsilylchloride in THF at 0 °C as the best condition to furnish the desired product in quantitative yields.[12] A reasonable diastereoselectivity of erythro/threo (**7a**/**7b**, 3/7) was obtained and the selectivity towards diastereomer **7b** was quite favorable. It was observed that these two diastereomers could be easily separated by column chromatography.[13]

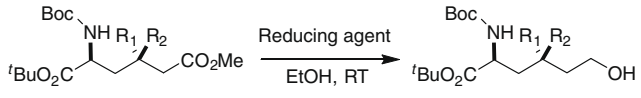
The enantiomerically pure Reformatsky products **7a** and **7b** serve as useful precursors for native chemical ligation. The major product (**7b)** of Reformatsky reaction was protected with various protecting groups like, TBDMSCl (**8a**, 80%), TBDPSCl (**8b,** 89%), and TPSCl (**8c,** 75%), as shown in Scheme 3. The selective reduction of methyl ester to the corresponding terminal alcohol was initially attempted by using different reducing agents like $LiAlH_4$, DIBAL-H, $NaBH_3CN$, and $NaBH_4$ (Table 1, entries 3–6). Among them, $NaBH_4$ in ethanol at room temperature was found to be superior to other reducing reagents in terms of yields (Table 1, entry 6). With the optimized reaction conditions in hand, we decided to investigate the influence of reducing reagent ($NaBH_4$) on the different protecting groups (entries 6–10). In all cases TBDPS-protected amino acid treated with $NaBH_4$ in ethanol gave the best result with 92% yield (entry 6). The stereochemistry of this compound was assigned by analogy to the similar substrate.[14]

Having the optimized reaction conditions, TBDPS-protected substrate **9** was treated with methanesulfonyl chloride and DIPEA



Scheme 3. Reagents and conditions: (i) general procedure for **8b**, TBDPSCl, imidazole, DCM, rt, 89%.

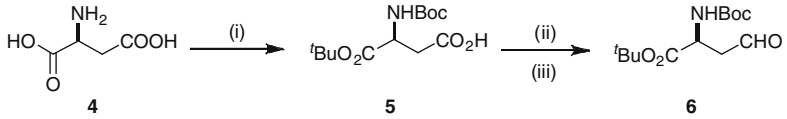
Table 1
Optimization of reaction conditions



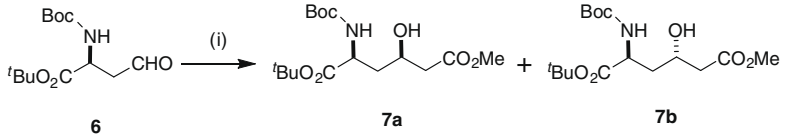
No.	R_1	R_2	Reducing agent	Solvent	Yield (%)
1	OTBDMS	H	$LiAlH_4$	Et_2O	—
2	OTBDMS	H	DIBAL-H	THF	23
3	OTBDMS	H	$NaBH_3CN$	EtOH	36
4	OTBDMS	H	$NaBH_4$	MeOH	53
5	OTBDMS	H	$NaBH_4$	EtOH	70
6	OTBDPS	H	$NaBH_4$	EtOH	92
7	OTPS	H	$NaBH_4$	EtOH	65
8	OH	H	$NaBH_4$	EtOH	70
9	H	OTBDMS	$NaBH_4$	EtOH	68
10	H	OTBDPS	$NaBH_4$	EtOH	85

using standard procedure to obtain mesylated product which was converted into azide using NaN_3 in DMF at 80 °C (Scheme 4). The azide **10** was subjected to Pd/C catalyzed hydrogenation under H_2 atmosphere at room temperature to form amine, which was easily protected with Cbz group using the standard procedure. The last part of the synthesis of protected 4-mercapto-L-lysine consists of introducing thiol unit into lysine chain. After deprotection of the silyl ether **11** with tetra-butylammonium fluoride (TBAF), the secondary alcohol of amino acid **12** was mesylated followed by nucleophilic substitution of thioacetate to afford **13** in good yield.[15] Saponification and subsequent protection of **13** with *S*-methyl methanethiosulfonate (MMTS) furnished **14**, which was then treated with TFA to get unprotected aminoacid (**15**) in good yields. Further protection with Boc anhydride delivered the desired amino acid **16**.[16] The unprotected amino acid **16** is useful intermediate for dual native chemical ligation.[17]

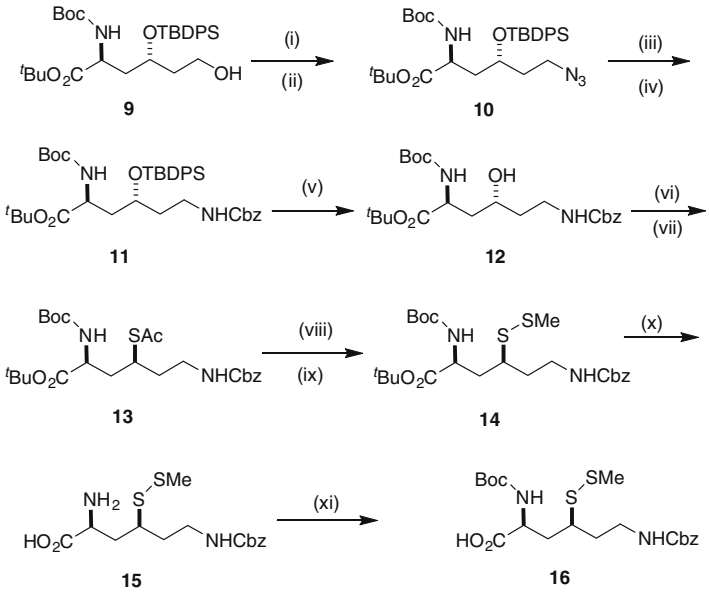
Accordingly, (2*S*,4*S*)-4-hydroxy-L-lysine **12** was useful precursor to generate 4-substituted-L-lysine derivatives (Fig. 2) by using different nucleophiles, such as F^-, Br^-, and N_3^-. Similarly, enantiomerically pure minor product (**7a)** of Reformatsky reaction serves as a



Scheme 1. Reagents and conditions: (i) Ref. 8; (ii) EtSH, DCC, DMAP, DCM, rt, 95%; (iii) Et_3SiH, 10% Pd/C, DCM, rt, 85%.



Scheme 2. Reagents and conditions: (i) methyl bromoacetate, Zn, TMSCl, THF, 0 °C, 92%.



Scheme 4. Reagents and conditions: (i) methanesulfonyl chloride, diisopropyl ethylamine (DIPEA), 0 °C; (ii) NaN_3, DMF, 80 °C, two steps 83% yield; (iii) Pd/C, ethyl acetate, quantitative yield; (iv) CbzCl, $NaHCO_3$, dioxane:water (2:1), 0 °C, 81%; (v) TBAF, THF, 0 °C, 77%; (vi) methanesulfonyl chloride, diisopropyl ethylamine (DIPEA), 0 °C; (vii) potassium thioacetate, DMF, 40 °C, two steps 70% yield; (viii) NaOH, MeOH, rt; (ix) *S*-methyl methanethiosulfonate (MMTS), triethylamine, CH_2Cl_2, rt, 50% (two steps); (x) 95% TFA, H_2O, rt; (xi) Boc_2O/TEA, MeOH, rt, 78% over two steps.

useful precursor for another potentially useful substrate for native chemical ligation. Synthesis of these analogues is currently under way in our laboratory.

In summary, a practical and concise synthesis of amino acid (2*S*,4*S*)-4-mercapto-L-lysine has been developed starting from commercially available L-aspartic acid. The synthesis involves diastereoselective Reformatsky reaction as a key step to form functionalized L-lysine derivatives. We believe that this approach allows a direct access to 4-hydroxy-L-lysine derivatives that offer some interesting biologically active molecules and useful building blocks for native chemical ligation.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.107.

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12. Selected reference on Reformatsky reaction: (a) Furstner, A. *Synthesis* **1989**, 571; (b) Rathka, M. W. *Org. React.* **1975**, *22*, 423; (c) Shriner, R. L. *Org. React.* **1942**, *1*, 1.
13. *(2S,4R)-1-tert-Butyl 6-methyl 2-(tert-butoxy carbonyl amino)-4-hydroxy hexanediote* (**7a**)/*(2S,4S)-1-tert-butyl 6-methyl-2-(tert-butoxy carbonyl amino)-4-hydroxy hex anediote* (**7b**): To a cooled (0 °C) solution of aldehyde (0.50 g, 1.83 mmol) in THF (7.32 mL, 0.25 M) was added dropwise a solution of Reformatsky reagent (4.57 mL, 4.57 mmol) (the experimental procedure for Reformatsky reagent given below) over a period of 15 min and the reaction mixture was stirred for 2 h. The reaction was quenched by the addition of saturated, aqueous ammonium chloride solution and extracted three times with ethyl acetate. The combined organic layers were then washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, then concentrated in vacuo. The residue was purified by silica gel flash column chromatography using ethyl acetate/hexane (20:80) as eluent. R_f (**7a**) = 0.28; R_f (**7b**) = 0.34. Combined yield 92% (colorless solid). **7a** [2*S*,4*R*] diastereomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 5.38 (br s, 1H, NH), 4.20–4.18 (m, 2H, *CH*–NH, C*H*–OH), 3.68 (s, 3H, CH_3), 3.24 (br s, 1H, OH), 2.54–2.44 (m, 2H, CH_2–CO_2Me), 1.94–1.86 (m, 2H, CH_2), 1.44 (s, 9H, 3CH_3), 1.41 (s, 9H, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ in ppm = 172.8 (C=O), 171.5 (C=O), 155.5 (C=O), 82.0 (C), 79.8 (C), 65.2 (*C*H–OH), 51.9 (CH_3), 51.7 (*C*–NH), 41.0 (CH_2), 38.8 (CH_2), 28.3 (3CH_3), 27.9 (3CH_3); IR ($CHCl_3$): $\bar{\nu}$ = 3429, 3018, 2980, 2401, 1724, 1367 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{16}H_{30}NO_7$: 348.2022 $[M]^+$; found: 348.2021. Compound **7b** [2*S*,4*S*] diastereomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 5.42 (d, *J* = 7.9 Hz, 1H, NH), 4.37–4.33 (m, 1H, *CH*–OH), 4.21 (d, *J* = 3.5 Hz, 1H, CH-NH), 4.07 (br s, 1H, OH), 3.68 (s, 3H, CH_3), 2.55 (dd, *J* = 15.7 and 8.1 Hz, 1H, CH–CO_2Me), 2.40 (dd, *J* = 15.7 and 4.7 Hz, CH–CO_2Me), 1.89–1.84 (m, 1H), 1.61–1.54 (m, 1H), 1.44 (s, 9H, 3CH_3), 1.41 (s, 9H, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ in ppm = 172.1 (C=O), 171.6 (C=O), 156.7 (C=O), 82.3 (C), 80.4 (C), 64.4 (*C*H–OH), 51.7 (CH_3), 51.2 (*C*H–NH), 41.2 (CH_2), 40.6 (CH_2), 28.2 (3CH_3), 27.9 (3CH_3); IR ($CHCl_3$): $\bar{\nu}$ = 3425, 3018, 2981, 2399, 1730, 1369 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{16}H_{30}NO_7$: 348.2022 $[M]^+$; found: 348.2033.*Preparation of Reformatsky reagent (1 M solution in THF)*: A flame-dried two-necked flask fitted with a reflux condenser and septum was charged with zinc dust (0.47 g, 7.18 mmol), trimethylchlorosilane (TMSCl) (0.11 mL, 0.98 mmol), and dry THF (3.50 mL), then heated to reflux with stirring under nitrogen for 15 min. The flask was then removed from the heat and methyl bromoacetate (0.60 mL, 6.53 mmol) in dry THF (3.50 mL) was added via syringe at such a rate as to maintain gentle reflux. At this stage the reaction was stopped to allow the coarse Zn particles to settle down. The upper green color solution (Reformatsky reagent, 1 M solution in THF) can be readily used for further steps.
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15. Representative experimental procedures for compound **14** *(2S, 4S)-tert-Butyl-4-(acetylthio)-6-(benzyloxy carbonylamino)-2-(tertbutoxy carbonyl amino) hexane –dioate*: Compound **12** (0.05 g, 0.07 mmol) was dissolved in dry THF (1.5 mL, 0.05 M) and cooled to 0 °C. 1 M solution of TBAF (0.11 mL, 0.11 mmol) was introduced via syringe at the same temperature. The reaction mixture was stirred for 8 h at 0 °C and quenched with saturated ammonium chloride solution. Evaporation of the solvent gave a residue, which was dissolved in

ethyl acetate. The solution was washed with water and brine. The organic layer was dried over sodium sulfate and concentrated in vacuo, which was purified by silica column chromatography to give desired alcohol (0.025 g, 77%) as a colorless oil. Then, 0.08 mL (0.44 mmol) of diisopropyl ethylamine (DIPEA) was added to a solution of 0.10 g (0.22 mmol) of above alcohol in 2.2 mL (0.1 M) of dichloromethane at room temperature. The solution was cooled to 0 °C, and 0.02 mL (0.27 mmol) of methanesulfonyl chloride was added. The reaction mixture was allowed to reach room temperature and stirred for 2 h. The reaction mixture was quenched with saturated ammonium chloride solution at 0 °C and diluted with dichloromethane. The layers were separated, the aqueous layer was extracted with dichloromethane two times, and the combined organic extract was washed with water and brine. Drying over anhydrous sodium sulfate and removal of solvent under vacuum resulted in a colorless oily residue which was dissolved in DMF (4.4 mL, 0.05 M). A total of 0.75 g (0.66 mmol) of potassium thioacetate was added to the solution, which was heated to 40 °C for 8 h. After allowed to reach room temperature, water was added to the solution, which was extracted twice with ethyl acetate. The crude product was purified by column chromatography on silica gel to give desired product **14** (0.04 g, 70%) as a pale brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 7.34–7.28 (m, 5H, Ph), 5.46 (br s, 1H, NH), 5.23 (d, J = 8.0 Hz, 1H, NH), 5.07 (dd, J = 14.8 and 12.5 Hz, 2H, CH_2-Ph), 4.26 (dd, J = 13.6 and 7.8 Hz, 1H, C*H*–NH), 3.66–3.59 (m, 1H, C*H*–S), 3.39 (dd, J = 13.0 and 5.4 Hz, 1H, C*H*$_2$–NH), 3.15–3.10 (m, 1H, C*H*$_2$–NH), 2.31 (s, 3H, CH_3), 2.03 (dd, J = 13.4 and 6.5 Hz, 2H, CH_2), 1.90–1.82 (m, 1H, C*H*$_2$), 1.68–1.59 (m, 1H, CH_2) 1.44 (s, 9H, 3C*H*$_3$), 1.40 (s, 9H, 3C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ in ppm = 195.9 ($COCH_3$), 171.1 (C=O), 156.4 (C=O), 155.6 (C=O), 136.7 (Ph), 128.4 (Ph), 128.1 (Ph), 127.9 (Ph), 82.4 (C), 80.1 (C), 66.5 (CH_2-Ph), 51.9 (*C*H–NH), 39.7 (*C*H$_2$–NH), 38.2 (CH_2), 38.2 (CH_2), 33.7 (CH–S), 33.7 (CH_3) 28.2 (3CH_3), 27.9 (3CH_3); IR ($CHCl_3$): $\bar{\nu}$ = 3431, 3018, 2399, 1645, 2088, 1637, 1215 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{25}H_{39}N_2O_7S$: 511.2478 $[M]^+$; found: 511.2480.

16. The white powder of Boc-lys(SSMe, Cbz)-OtBu **14** was dissolved in 0.5 mL of 95% TFA. After 1 h at room temperature, TFA was removed by evaporation to get unprotected amino acid **15** in quantitative yield. The residue was dissolved in 1 mL of methanol/H_2O mixture (3:1). pH was adjusted to 8.0 with Et_3N. 0.02 mL of Boc_2O was added to react with **15** for 3 h at room temperature. The mixture was subjected to semi-preparative HPLC isolation on C18 column. Boc-Lys(SSMe, Cbz)-OH **15** (7 mg, 78% yield) was isolated. ^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 7.34–7.33 (m, 5H, Ph), 5.33 (br s, 1H, NH), 5.29 (d, J = 7.2 Hz, 1H, NH), 5.08 (s, 2H, CH_2-Ph), 4.43 (b, 1H, C*H*–NH), 3.38 (b, 2H, C*H*$_2$), 2.92–2.86 (m, 1H, C*H*), 2.38 (s, 3H, CH_3), 2.17–2.03 (m, 2H, C*H*$_2$), 1.87–1.83 (m, 2H, C*H*$_2$) 1.39 (s, 9H, 3C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ in ppm = 175.3 (C=O), 156.7 (C=O), 156.0 (C=O), 136.4 (Ph), 128.4 (Ph), 128.2 (Ph), 128.1 (Ph), 80.8 (C), 66.8 (CH_2-Ph), 51.4 (CH–NH), 44.7 (*C*H$_2$–NH), 39.1 (*C*H$_3$–S), 38.5 (CH_2), 32.6 (CH_2), 28.2 (3CH_3), 24.0 (CH-S). IR ($CHCl_3$): $\bar{\nu}$ = 3440, 3022, 2399, 1650, 1640 cm^{-1}. HRMS (ESI): *m/z*: calcd for $C_{20}H_{30}N_2O_6S_2Na$: 481.1443; $[M+Na]^+$; found 481.1446.

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