# A new route for the synthesis of 1-amino-3,6,9,12-tetraoxapentadecan-15-oic acid

**Xuan Wu[a], Xi Zong[a] and Min Ji[b]***

[a]*School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 210000, P.R. China*
[b]*School of Biological Science and Medical Engineering & Collaborative Innovation Center of Suzhou Nano Science and Technology, Southeast University, Suzhou, 215123, P.R. China*

1-Amino-3,6,9,12-tetraoxapentadecan-15-oic acid **8** was synthesised from tetraethylene glycol through a 7 step sequence including esterification, mesylation, azide substitution with subsequent reduction followed by hydrolysis. The structure of product **8** was identified by $^{1}H$ and $^{13}C$ NMR spectroscopy, elemental analysis and electrospray ionisation mass spectrometry (ESI-MS). All reaction conditions were optimised and easy to control. The key advantages of this process are the high yields of products and a new route to synthesise 1-amino-3,6,9,12-tetraoxapentadecan-15-oic acid.

**Keywords:** PEG, linker, synthesis method, protecting group

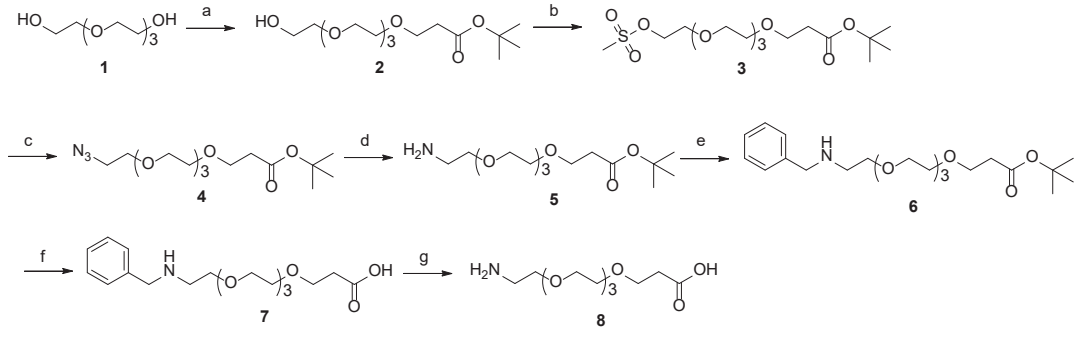
Polyethylene glycol (PEG) is a composed of ethylene glycol units and can range in molecular weight from a few hundred to several tens of hundreds. It is a biocompatible water-soluble polymer[1] due to its low toxicity, low immunogenicity and good solubility in both aqueous and organic solvents.[2–5] It also can be excreted through the kidney and will not be accumulated in the body.[6] More importantly, when PEG couples with other modifications, its many attractive properties would be transferred to the compounds.[7–9] Therefore it is widely used as a carrier system for biological proteins.[10,11]

Hybrid formation of bioactive materials with PEG is the focus of many studies on drug delivery systems.[12] Excellent biocompatibility of PEG has resulted in its applications in medicine and biotechnology, and some have received FDA approval.[13–17] In the past several years, a variety of micromolecule PEG derivatives which contain both carboxyl and amino functions have been used as linkers to connect sialyl LewisX amine with protected spacer-equipped asparagines.[18] Currently, PEGs are also widely used in the field of prodrug research, because it can improve not only tumour uptake[19] but also excretion kinetics of $^{125}I$- and $^{18}F$-labeled c(RGDyK) and $^{64}Cu$-labeled $E[c(RGDyK)]_2$.[20–23]

These results indicate that PEG offers many interesting opportunities in the development of novel drug-carrier systems and has also been used in the synthesis of many compounds as a linker. Compound **8** and PEG have the same fragment. Besides, compound **8** contains carboxyl and amino functions which can easily react with other compounds. Thus, the compound **8** is attracting more and more attention and several methods for its synthesis have been developed.[24–26] In spite of their potential utility, many of these reported syntheses have drawbacks such as harsh reaction conditions, unsatisfactory yields, prolonged reaction times, cumbersome product isolation procedures, polar, volatile and hazardous organic solvents, which limit their application. Therefore, there is continuous demand for the development of a novel environmentally friendly synthetic method. Herein, we report a simple and highly efficient method for the preparation of compound **8**. In our route, compound **8** and two useful intermediates (compounds **5** and **7**) can be easily obtained. Compared with other synthetic routes,[24–26] the yield from our approach is higher and the reaction conditions are easier to control. More importantly all the materials are readily available and cost effective.

## Results and discussion

As shown in Scheme 1, the spacer amino acid was synthesised from tetraethylene glycol and its base catalysed conjugate addition to *tert*-butyl acrylate to yield **2**. After mesylation to afford **3**, the latter was subjected to nucleophilic displacement by treatment with sodium azide in DMF to give the azido ester **4** in good yield (65%) following chromatography. Hydrogenation of the azido group in **4** furnished the spacer amino acid ester **5**. Finally, **7** was obtained through hydrolysis of the carboxylic ester following benzylation and protection of the amino group




**Scheme 1** Reagents and conditions: (a) Na, *tert*-butyl acrylate, THF, r.t., 24 h, 77%; (b) $MeSO_2Cl$, $Et_3N$, $CH_2Cl_2$, 0 °C, 15 h, 92%; (c) $NaN_3$, DMF, r.t., 2 d, 65%; (d) Raney nickel, $H_2$, *i*-PrOH, r.t., 12 h, 93%; (e) BnBr, $CH_2Cl_2$, r.t., 18 h, 72%; (f) LiOH (4 mol $L^{-1}$), MeOH, reflux, 5 h, 93%; (g) Pd/C, $H_2$, MeOH, r.t., 15 h, 94%.

* Correspondent. E-mail: zhiming@cczu.edu.cn

as in **6**. Subsequent hydrogenolysis of the *N*-benzyl group provided **8** in 94% yield. In our route, we can easily obtain compound **8** and two useful intermediates (compounds **5** and **7**). These two intermediates have a protecting group at the carboxyl or amino function. PEG can be replaced by one of compounds **5**, **7**, **8** according to the actual situation. The reaction sequence is applicable to other chain lengths and analogues of **1** to access other derivatives of compound **8**.

The structure of **8** was confirmed on the basis of spectroscopic data, particularly its $^1$H NMR and MS data. In the $^1$H NMR spectrum of **8**, the singlet signal at 11.21 ppm was due to the –COOH proton. The singlet at 8.88 ppm was due to $NH_2$ function. Two triplets at 2.94 and 2.45 ppm correspond to the N–$CH_2$ and –$CH_2$COO– protons respectively. In addition, the signals in $^{13}$C NMR also proved the existence of –$NH_2CH_2$ and –COOH functions. In ESI-MS spectrum, the calculated *m/z* $[M + H]^+$ of **8** was 266.15254, and the *m/z* found was 266.15589.

## Conclusion

In conclusion, an efficient and simple method for the synthesis of 1-amino-3,6,9,12-tetraoxapentadecan-15-oic acid **8** under mild conditions was reported. The main advantages of this method are the new route to synthesise **8** and the high yields of products.

## Experimental

All chemicals were of analytical reagent grade and purchased from commercial sources, which were used directly without further purification. $^1$H and $^{13}$C NMR spectra were recorded in $CDCl_3$ solutions on a Bruker Avance 300 spectrometer; chemical shifts (δ) are reported in parts per million (ppm) relative to the internal standard tetramethylsilane (TMS). Electrospray ionisation mass spectrometry (ESI-MS) was obtained on a Finnigan MAT-95 Spectrometer.

tert-*Butyl 1-hydroxy-3,6,9,12-tetraoxapentadecan-15-oate* (**2**)

To dry tetraethylene glycol (17.2 mL, 0.10 mol) in dry tetrahydrofuran (100 mL), sodium (0.02 g, 0.87 mmol) was added. After 2 h, the sodium had dissolved and *tert*-butyl acrylate (4.35 mL, 0.03 mol) was added. The solution was stirred under exclusion of moisture for 24 h. After neutralisation with 1 M HCl (0.8 mL), the solvent was evaporated under reduced pressure. The residue was dissolved in brine (100 mL) and extracted three times with ethyl acetate (150 mL). The combined organic layers were washed with water (50 mL) and dried with $MgSO_4$. The solvent was evaporated under reduced pressure to afford **2** as clear, pale yellow liquid; 4.3 g, 77%; $^1$H NMR (300 MHz, $CDCl_3$): 3.77–3.52 (m, 18H), 3.06 (s, 1H), 2.41 (t, *J* = 5.8 Hz, 2H), 1.40 (s, 9H); ESI-MS *m/z*: $[M + H]^+$ 323.20252.

tert-*Butyl 1-mesyloxy-3,6,9,12-tetraoxapentadecan-15-oate* (**3**)

To a solution of **2** (2.0 g, 6.2 mmol) in dry $CH_2Cl_2$ (30 mL) and $Et_3N$ (2.1 mL, 15 mmol) of at 0 °C, methanesulfonyl chloride (1.0 mL, 13 mmol) was added dropwise within 15 min. After stirring for 15 h at 0 °C, filtration through Hyflo and washing with water and brine, the $CH_2Cl_2$ layer was dried with $MgSO_4$, filtered, and concentrated *in vacuo* to afford **3** as dark brown oil; 2.3 g, 92%; $^1$H NMR (300 MHz, $CDCl_3$): 3.69–3.52 (m, 18H), 3.14 (s, 3H), 2.44 (t, *J* = 6.1 Hz, 2H), 1.40 (s, 9H); ESI-MS *m/z*: $[M + H]^+$ 401.18003.

tert-*Butyl 1-azido-3,6,9,12-tetraoxapentadecan-15-oate* (**4**)

Sodium azide (0.82 g, 12.5 mmol) was added to solution of the mesylate **3** (2.5 g, 6.2 mmol) in DMF (30 mL). The mixture was stirred under exclusion of moisture for 2 d at room temperature. The reaction mixture was filtered and the residue was partitioned between EtOAc and $H_2O$, The aqueous phase was extracted with EtOAc, and the combined organic phases were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel; 30% EtOAc–PE) to afford **4** as pale yellow oil; 1.4 g, 65%; $^1$H NMR (300 MHz, $CDCl_3$): 3.76–3.53 (m, 16H), 3.33 (t, *J* = 5.9 Hz, 2H), 2.44 (t, *J* = 6.1 Hz, 2H), 1.40 (s, 9H); ESI-MS *m/z*: $[M + H]^+$ 348.20899.

tert-*Butyl 1-amino-3,6,9,12-tetraoxapentadecan-15-oate* (**5**)

To a solution of **4** (1.0 g, 2.88 mmol) in isopropanol (20 mL) freshly prepared Raney nickel (0.7 g, pH 9) was given. Air was exchanged for argon and then for hydrogen. After hydrogenation for 12 h, the catalyst was filtered off through Hyflo and washed with isopropanol (50 mL). The solvent was evaporated *in vacuo* to afford **5** as pale yellow oil; 0.86 g, 93%; $^1$H NMR (300 MHz, $CDCl_3$): 3.69–3.53 (m, 14H), 3.45 (t, *J* = 5.8 Hz, 2H), 2.64 (t, *J* = 5.8 Hz, 2H), 2.47 (t, *J* = 6.1 Hz, 2H), 1.42 (s, 9H); ESI-MS *m/z*: 322.21849 $[M + 1]^+$.

tert-*Butyl 1-phenyl-5,8,11,14-tetraoxa-2-azaheptadecan-17-oate* (**6**)

To a solution of **5** (0.4 g, 1.22 mmol) in dry $CH_2Cl_2$ (15 mL) at 0 °C, benzyl bromide (0.34 g, 2.0 mmol) was added dropwise within 10 min. After stirring for 18 h at r.t., the reaction mixture was then quenched by the addition of saturated aqueous $NaHCO_3$ (20 mL). The resulting aqueous solution was extracted with $CH_2Cl_2$ (3 × 50 mL) and the organic extracts were combined, and dried over $Na_2SO_4$. The residue was purified by flash chromatography (silica gel; 20% EtOAc–PE) to afford **6** as pale yellow oil; 0.36 g, 72%; $^1$H NMR (300 MHz, $CDCl_3$): 7.36–7.17 (m, 5H), 3.68–3.48 (m, 18H), 2.66 (t, *J* = 5.9 Hz, 2H), 2.45 (t, *J* = 6.4 Hz, 2H), 1.43 (s, 9H); ESI-MS *m/z*: $[M + H]^+$ 412.26544.

*1-Phenyl-5,8,11,14-tetraoxa-2-azaheptadecan-17-oic acid* (**7**)

Compound **6** (0.3 g, 0.73 mmol) was added to an aqueous solution of LiOH (4 mol/L, 0.4 mL) and THF (15 mL). The solution was heated to reflux for 5 h. After concentration, the residue was partitioned between EtOAc and water. The pH of the combined aqueous phase was adjusted to 1 with 10% HCl, and then washed with EtOAc. The EtOAc layer was washed with brine and then dried over anhydrous $Na_2SO_4$ and concentrated to give product **7** as pale yellow oil; 0.24 g, 93%; $^1$H NMR (300 MHz, $CDCl_3$): 12.21 (s, 1H), 7.72–7.44 (m, 5H), 3.59–3.43 (m, 18H), 3.01 (t, *J* = 5.9 Hz, 2H), 2.45 (t, *J* = 6.1 Hz, 2H); ESI-MS *m/z*: $[M + H]^+$ 356.20284.

*1-Amino-3,6,9,12-tetraoxapentadecan-15-oic acid* (**8**)

A mixture of compound **7** (200 mg, 0.56 mmol), 10% Pd–C catalyst (64 mg, 0.06 mmol) in MeOH (10 mL) was stirred under a hydrogen atmosphere at room temperature and atmospheric pressure until the absorption of hydrogen ceased. After the Pd–C catalyst was filtered off, the solvent was evaporated *in vacuo* to afford **8** as pale yellow oil; 140 mg, 94%; $^1$H NMR (300 MHz, $CDCl_3$): 11.21 (s, 1H), 8.88 (s, 2H), 3.64–3.50 (m, 18H), 2.94 (t, *J* = 6.1 Hz, 2H), 2.45 (t, *J* = 6.0 Hz, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$): 172.80, 73.52, 70.59, 70.52, 70.33, 70.31, 66.88, 41.54, 34.81; ESI-MS *m/z*: $[M + H]^+$ 266.15589; Anal. calcd for $C_{11}H_{23}NO_6$: C, 49.80; H, 8.74; N, 5.28; found: C, 49.50; H, 8.59; N, 5.31%.

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