Chinese Chemical Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

Acid dissociation constants and cytotoxicity test of a series of omega-aminoalkyl phosphates

Fu-Hua Sun, Yuan-Zhu Long, Xiao-Yong Zhou, Yi-Rou Jiang, Xing-Yi Xie *

Department of Polymeric Biomaterials and Artificial Organs, College of Polymer Science and Engineering, Sichuan University, Chengdu 610065, China

ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form 11 March 2016
Accepted 16 March 2016
Available online xxx

Keywords:
Aminoalkyl phosphates
Dissociation constant
Cytotoxicity
Odd–even effect
Hydroxyapatite

ABSTRACT

We synthesised a series of ω-aminoalkyl sodium hydrogen phosphates (AAP-*n*-Na, $n = 3, 4, 5, 6$, purity > 99%), which have potential applications as bioactive cosmetic ingredients and surface modifiers of bone minerals (*i.e.* hydroxyapatites). Results from Fourier transformed infrared (FTIR), nuclear magnetic resonance (NMR) and high resolution mass spectroscopy, and elemental analysis all matched their chemical structures. The acid dissociation constant (p*K*a's) of each AAP-*n* (acid form of AAP-*n*-Na, $n = 2$–6) were measured by potentiometric titration, showing a general increasing trend with an increase in the chain length of AAP-*n*. However, the pKa_3 constant, which corresponds to the deprotonation of the ammonium group in AAP-*n*-Na, displayed an unusual decrease when *n* = even. This odd–even effect can be explained by the pairwise self-association of AAP-*n*-Na molecules in water where intermolecular hydrogen bonding in case of *n* = even is weaker than that in case of *n* = odd. All AAP-*n*-Na at concentrations up to 0.1% (w/v) were non-toxic to L929 fibroblasts and MG 63 osteoblast-like cells in terms of cell growth and morphology. These basic data were important for applications of AAP-*n* and their salts in biomedical engineering.

1. Introduction

Nowadays, ω-aminoalkyl dihydrogen phosphates H_2N–$(CH_2)_n$–$OP(O)(OH)_2$ (referred to as AAP-*n* thereafter) and their salts have found limited biomedical applications despite the fact that they possess functional amino and phosphate groups present in many biological molecules, like proteins and nucleic acids. AAP-2, a phospholipid moiety, has been used to stabilise apatite colloid with potential for cellular drug delivery [1]. AAP-3 (or its salt) is an active cosmetic ingredient promoting collagen biosynthesis in the skin, as shown in a US patent [2]. AAP-6 has been condensed with biological molecules like biotin [3] and uridine 5′-monophosphate [4] to form various bioconjugates.

The synthesis of AAP-*n* involves O-selective phosphorylation of corresponding amino alcohols. Phosphorus oxychloride ($POCl_3$) is not suitable because it reacts with both hydroxyl and amino groups. Only AAP-3 has been synthesised using this phosphorylation reagent, which reacts with 3-aminopropanol forming a cyclic phosphoramidate chloride initially, followed by a selective hydrolysis of the P(O)–N bond in the six-member ring [2]. A more universal synthesis has been achieved using phosphoric acid [3–6] or pyrophosphoric acid [7] as the phosphorylation reagent. However, a high temperature (140–250 °C), a long reaction time (18–40 h) and a high vacuum (below 50 mmHg) are usually required for these methods.

The inconvenient synthesis may account for the limited availability of AAP-*n*. To the best of our knowledge, only AAP-2 is commercially available (*e.g.* from Sigma–Aldrich). We recently synthesised a series of ammonium salts of AAP-*n* ($n = 3, 4, 5, 6$) at mild temperatures (0–25 °C) using $POCl_3$ as the phosphorylation reagent [8]. The key is to protect the amino group of each amino alcohol with a fluorenylmethyloxycarbonyl (Fmoc) group prior to phosphorylation. We further adopted these ammonium salts as dispersing agents to synthesise hydroxyapatite hydrocolloids [9], showing an increase in the aspect ratio of the colloidal particles with an increase in the carbon number of the dispersant.

In this study, we modified our previous synthesis [8], forming a monosodium salt of each AAP-*n* ($n = 3$–6, referred to as AAP-*n*-Na thereafter) which are easy to purify *via* recrystallisation. This simple synthesis resulted in highly pure AAP-*n*-Na (purity over 99%), making it possible to finely characterise their chemical structures and compositions. Based on this, the p*K*a constants of

* Corresponding author.
E-mail address: xiexingyi@scu.edu.cn (X.-Y. Xie).

http://dx.doi.org/10.1016/j.cclet.2016.03.029

AAP-*n* series and the cytotoxicity of their sodium salts (AAP-*n*-Na) were determined and reported for the first time. We believe these basic data are important for further research into and applications of AAP-*n* and their salts in biomedical engineering, such as the functionalisation of hydroxyapatite, which is the mineral phase of bone.

2. Experimental

2.1. Materials

O-phosphorylethanolamine (*i.e.* AAP-2, >98%, TCI, Japan) was used as a control. 3-Amino-1-propanol (>98.5%, J & K Scientific Ltd., Beijing, China), 4-amino-1-butanol (>98%, Chengdu Best Reagent Co., Ltd., Chengdu, Sichuan, China), 5-amino-1-pentanol (>96%, Alfa Aesar, USA) and 6-amino-1-hexanol (>97%, also from J & K Scientific Ltd.) served as amino alcohols (AC-*n*, $n = 3–6$). Fluorenylmethyloxycarbonyl chloride (Fmoc-Cl, 99%, Asta Tech, Chengdu, Sichuan, China) and $POCl_3$ (>98%, Kelong Chemical, Chengdu, Sichuan, China) were used as amino-protecting and phosporylating agents, respectively.

2.2. Synthesis of AAP-n-Na

As shown in Fig. 1, the whole synthesis involved three steps, *i.e.*, protecting the amino group, phosphorylating the hydroxyl group and removing the protecting group. The first two steps were described previously [8]. In the third step, each Fmoc-AAP-*n* (0.025 mol) was dissolved in 30 mL of *N*,*N*-dimethylformamide (DMF), into which 150 mL of piperidine/DMF (1:4, v/v) was slowly dripped. After magnetically stirring for 2 h, a resulting white precipitate (which was a mixture of AAP-*n* and its piperidine salt with a molar ratio of 1:1, see Fig. S1 in Supporting information) was obtained by filtration, and then washed with 30 mL × 3 of ethyl acetate. The washed precipitate was dissolved in 30 mL of water, and the pH was adjusted to about 11.2 using a solution of 0.5 mol/L NaOH. The water phase was extracted with 20 mL × 5 of chloroform. Each extraction proceeded for at least 0.5 h under vigorous stirring to allow piperidine to enter into the organic phase. Afterwards, the pH of the water phase was adjusted to 8.6–8.9 using 0.5 mol/L HCl prior to the addition of 20 mL of ethanol. The mixture was rested at −18 °C for 15 h to crystallise AAP-*n*-Na. Finally, the product was recrystallised the same way, and dried at 60 °C for 10 h.

2.3. Structural characterisation of AAP-n-Na

The Fourier transformed infrared (FTIR) spectra were obtained on a Nicolet 560 IR spectrometer (Nicolet Instruments, USA) using KBr disks, with a resolution of 4 cm^{-1} between 400 cm^{-1} and 4000 cm^{-1}. The ^{1}H, ^{13}C and ^{31}P nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV II 400 MHz NMR spectrometer (Bruker Corp., Switzerland). The high-resolution mass spectra were collected on a Bruker maXis II time-of-flight mass spectrometer (Bruker Daltonics, USA).

The C, H and N contents in each AAP-*n*-Na were analysed on a Euro EA 3000 elemental analyser (Leeman Labs Inc., USA). The P and Na contents were measured on a VG PQ ExCell inductive coupled plasma (ICP) emission spectrometer (TJA Corp., USA), using diluted sample solutions with known accurate concentrations.

2.4. Potentiometric titration of AAP-n-Na

We performed potentiometric titrations on each AAP-*n*-Na in water to determine its purity and the p*Ka* constants of corresponding AAP-*n*. Commercial AAP-2 was served as the control to assess the accuracy of the method. Typically, around 0.2 g AAP-*n*-Na (accurate mass recorded with an analytical balance) in 30 mL of water was titrated with 0.05 mol/L HCl and NaOH standard solutions, respectively. Their accurate concentrations were determined by titration with standard Na_2CO_3 (≥99.95%, Tianjin Zhiyuan Chemical Reagent Co., Ltd., Tianjin, China) or potassium biphthalate (≥99.95%, Kelong Chemical, Chengdu, Sichuan, China). All titrations were performed manually under magnetic stirring at room temperature (25 ± 0.5 °C). About 5 s after each titrant addition, a stable pH value of the analyte solution was measured with a Sartorius PB-10 pH metre (Sartorius AG, Germany). The detailed condition of each titration is shown in Table S1 in Supporting information.

Since the analyte mass (m_a) and the titrant concentration (C_t) are hard to maintain for all titrations (Table S1), it is inconvenient to compare the titration curves in the form of pH *vs.* real volume of

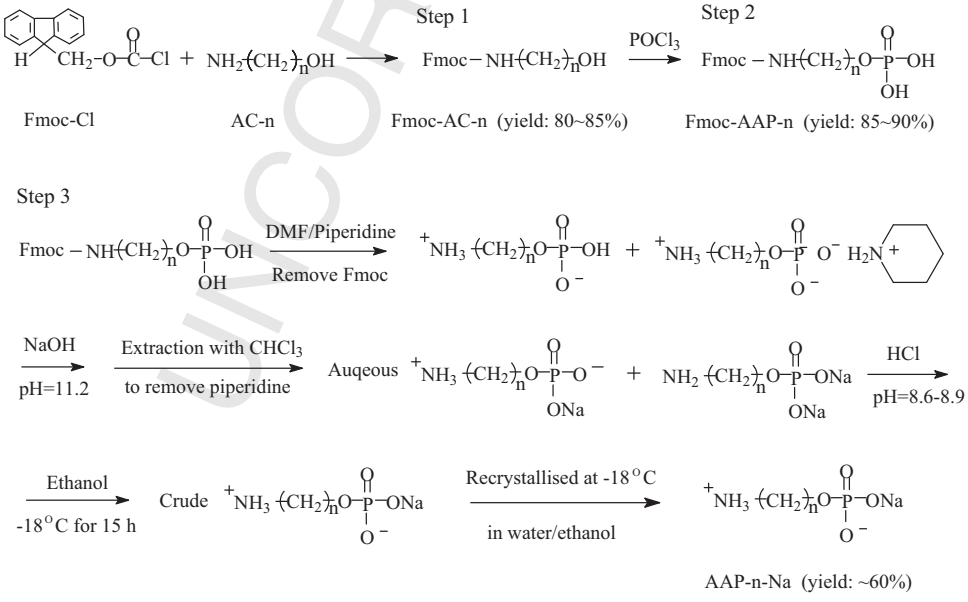

Fig. 1. Synthesis and purification of AAP-*n*-Na ($n = 3, 4, 5, 6$).

Please cite this article in press as: F.-H. Sun, et al., Acid dissociation constants and cytotoxicity test of a series of omega-aminoalkyl phosphates, Chin. Chem. Lett. (2016), http://dx.doi.org/10.1016/j.cclet.2016.03.029