Synthesis of 5-aminolevulinic acid (ALA) derivatives and their lipophilicity

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Abstract

Since ALA is a hydrophilic substance, its uptake into cells is low. Lipophilicity is, in general, one of the most effective properties for pharmacologically active agents to enhance their uptake by cells. Esterification is a well known method to increase the penetrability of the agent through plasma membranes. Many biochemical and clinical studies indicated that ALA alkyl esters can penetrate plasma membranes more readily and are enzymatically hydrolyzed to ALA, thus leading to the biosynthesis of PpIX in a large quantity in cells.

Here, we reported a simple reaction of ALA with alkanol to synthesize a series of methanesulfonate salts of ALA and derivatives successfully. NMR, elemental analysis confirmed their chemical structure, i.e. $CH_3SO_3H \cdot H_2NCH_2COCH_2$ -CH_2COOR (R=H, CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_8H_{17}, $C_{10}H_{21}$, $C_{12}H_{25}$, $C_{14}H_{29}$, $C_{16}H_{33}$, $C_{18}H_{37}$), labeled as C0-S, C1-S, C2-S, C3-S, C4-S, C5-S, C6-S, C8-S, C10-S, C12-S, C14-S, C16-S, C18-S, respectively.

Definitely, alkyl group improves the lipophilicity of these methanesulfonates, which was assessed by measuring the apparent partition coefficient (P) of the compounds between octanol and a PBS solution.

Key words : ALA derivatives, photosensitizer, synthesis, lipophilicity

1. Introduction

Photodynamic therapy (PDT) is a new clinical treatment of superficial tumors and age-related muscular degeneration. This technique involves the systemic administration of a photosensitive drug, by which singlet oxygen is generated after light irradiation, to produce oxidative damage to cells [1)-3)]. Photosensitizers play a crucial role in generating singlet oxygen in PDT. Porphyrin derivatives have been studied for decades as powerful photosensitizers [4)]. 5-aminolevulinic acid (ALA) as a protoporphyrin IX (PpIX) precursor is a frequently used compound for PDT. ALA is

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metabolized by mitochondria into the fluorescent molecule PpIX, which accumulates abnormally in tumor cells [5].

Since ALA is a hydrophilic substance, its uptake into cells is low [6),7]. Lipophilicity is, in general, one of the most effective properties for pharmacologically active agents to enhance their uptake by cells. Esterification is a well known method to increase the penetrability of the agent through plasma membranes [8),9]. There are then many biochemical and clinical studies on PDT using ALA alkyl esters [10)-15]. According to these studies, since ALA alkyl esters are more lipophilic than original ALA, they can penetrate plasma membranes more readily and are enzymatically hydrolyzed to ALA, thus leading to the biosynthesis of PpIX in a large quantity in cells [16].

Generally, the biochemical and clinical studies on ALA alkyl esters have used hydrochloride salts of ALA-related compounds [17]. Other salts are less reported. Here, a series of methanesulfonate salts of ALA alkylester with different alkyl length are synthesized by a simple method. Octanol-water partition coefficients of ALA and its esters methanesulfonates have been determined to obtain a parameter related to their lipophilicity.

2. Materials and Methods

2.1 Materials

ALA hydrochloride, alkyl alcohol (n= 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18), methanesulfonic acid, n-octanol and 0.1 M phosphate buffer (PBS) were purchased and used directly without further purification.

2.2 Characterization

NMR spectra were recorded on Bruker-AV 600MHz spectrometers. The 1H and 13C chemical shifts were obtained with DMSO as solvent and TMS as internal standard. Elemental analyses were carried out with a GmbH VarioEL V2.8 instrument.

The apparent partition coefficients (P) of ALA and its esters were determined in an octanol-buffer system at 21°C. The aqueous phase was a 0.1 M phosphate buffer (PBS) solution of pH 7.4. The PBS solution and the octanol were mutually saturated before use by shaking 300 mL of PBS with an equal quantity of octanol for 30 min. Twenty milligrams of the compound to be investigated were dissolved in 10 mL of the aqueous phase and an equal quantity of octanol was added. The mixtures were shaken for about 30 min and left for phase separation overnight at 4 °C. Absorption of both phases was measured with a UV-Vis absorption spectrometer (UV-2450, Shimadzu, Japan) at 269 nm (see Fig. 1(a)). The partition coefficients P were calculated according to:

$$P=c_{oct}/c_{PBS} = abs_{oct}/abs_{PBS}$$

where c_{oct} and c_{PBS} represent the solute concentrations in the organic and the aqueous phase, respectively, abs_{oct} is the absorption of the compound measured in the octanol and abs_{PBS} is the absorption in the PBS solution (see Fig. 1(b)). The use of low concentrations and storage at low temperatures impaired the formation of dimerization products. The absence of these products was confirmed by the absence of characteristic absorption bands in the absorption spectrum of

the measured solutions.

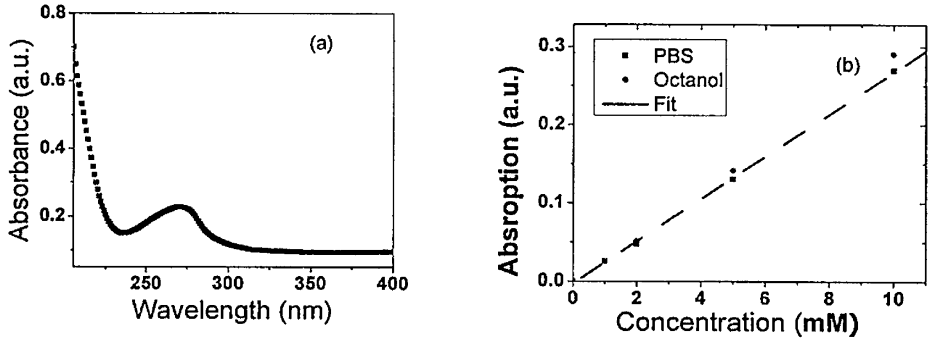


Fig. 1 (a). UV-visible absorption spectra of C4-S (10 mM) in PBS. (b). Absorption at 269 nm as a function of C4-S concentration in PBS and in octanol.

3. Results and Discussion

3.1 Synthesis of 5-aminolevulinic acid (ALA) derivatives

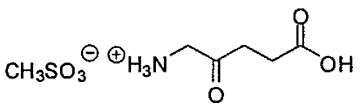
The reaction formula is as follows.

$$Cl^{\ominus}\,{}^{\oplus}H_3NCH_2COCH_2CH_2COOH(\text{ALA-HCl}) \xrightarrow[CH_3SO_3H\,/\,\text{solvent}]{C_nH_{2n+1}OH} CH_3SO_3^{\ominus}\,{}^{\oplus}H_3NCH_2COCH_2CH_2COOC_nH_{2n+1}(\text{ALA ester-}CH_3SO_3H)$$

Scheme 1 Synthesis of ALA ester methanesulfonate (n: 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, yield: 50 ~ 89%)

The chemical structure of these compounds is confirmed by ^{1}H- and ^{13}C-NMR spectra. The detailed data are described in the following.

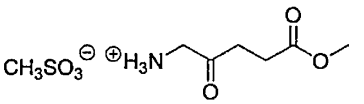
C0-S (5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 2.357 (s, 3H, CH_3), 2.488 (t, 2H, CH_2), 2.728 (t, 2H, CH_2), 3.954 (s, 2H, CH_2), 8.065 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 27.32, 34.36, 39.64, 46.80, 173.41, 202.86.

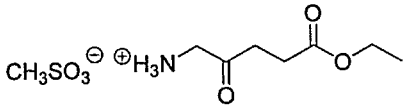
C1-S (methyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 2.353 (s, 3H, CH_3), 2,553 (t, 2H, CH_2), 2.795 (t, 2H, CH_2), 3.584 (s, 3H, CH_3), 3.965 (s, 2H, CH_2), 8.065 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 26.96, 34.24, 39.64, 46.75, 51.50, 172.47, 202.68.

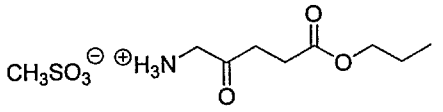
C2-S (ethyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 1.166 (t, 3H, CH_3), 2.369 (s, 3H, CH_3), 2.527 (t, 2H, CH_2), 2.779 (t, 2H, CH_2), 3.954 (s, 2H, CH_2), 4.028 (q, 2H, CH_2), 8.072 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 14.07, 27.23, 34.27, 39.59, 46.82, 60.11, 172.01, 202.67.

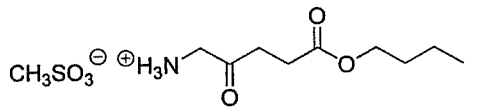
C3-S (propyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 0.879 (t, 3H, CH_3), 1.568 (m, 2H, CH_2), 2.345 (s, 3H, CH_3), 2.552 (t, 2H, CH_2), 2.791 (t, 2H, CH_2), 3.943 (s, 2H, CH_2), 3.962 (t, 2H, CH_2), 8.058 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 10.19, 21.46, 27.09, 34.22, 39.61, 46.72, 65.51, 172.02, 202.65.

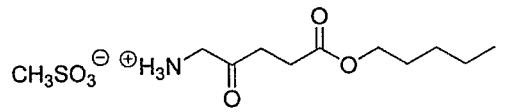
C4-S (butyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 0.878 (t, 3H, CH_3), 1.323 (m, 2H, CH_2), 1.536 (m, 2H, CH_2), 2.357 (s, 3H, CH_3), 2.527 (t, 2H, CH_2), 2.783 (t, 2H, CH_2), 3.956 (s, 2H, CH_2), 4.00 (t, 2H, CH_2), 8.060 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 13.55, 18.58, 27.14, 30.16, 34.26, 39.61, 46.80, 63.79, 172.07, 202.68.

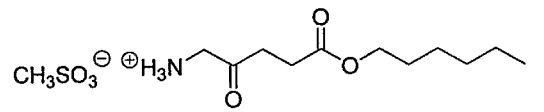
C5-S (pentyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 0.859 (t, 3H, CH_3), 1.268~1.285 (m, 4H, $2CH_2$), 1.550 (m, 2H, CH_2), 2.367 (s, 3H, CH_3), 2.533 (t, 2H, CH_2), 2.780 (t, 2H, CH_2), 3.954 (s, 2H, CH_2), 3.985 (t, 2H, CH_2), 8.068 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 13.82, 21.76, 27.16, 27.53, 27.79, 34.27, 39.59, 46.82, 64.09, 172.08, 202.65.

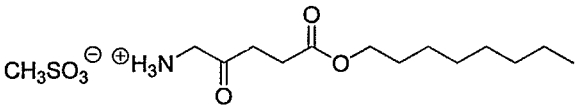
C6-S (hexyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 0.859 (t, 3H, CH_3), 1.241~1.307 (m, 6H, $3CH_2$), 1.548 (m, 2H, CH_2), 2.350 (s, 3H, CH_3), 2.541 (t, 2H, CH_2), 2.783 (t, 2H, CH_2), 3.959 (s, 2H, CH_2), 3.991 (t, 2H, CH_2), 8.058 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 13.83, 21.95, 24,97, 27.10, 28.03, 30.83, 34.23,39.64, 46.77, 64.08, 172.04, 202.65.

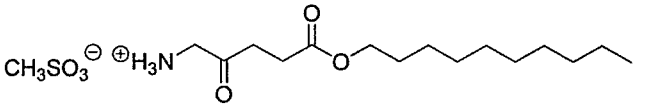
C8-S (octyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 0.854 (t, 3H, CH_3), 1.250~1.284 (m, 10H, $5CH_2$), 1.549 (m, 2H, CH_2), 2.345 (s, 3H, CH_3), 2.541 (t, 2H, CH_2), 2.784 (t, 2H, CH_2), 3.963 (s, 2H, CH_2), 3.9891 (t, 2H, CH_2), 8.059 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 13.88, 22.02, 25.30, 27.08, 28.05, 28.53, 28.58, 31.17, 34.21, 39.64, 46.78, 64.06, 172.01, 202.62.

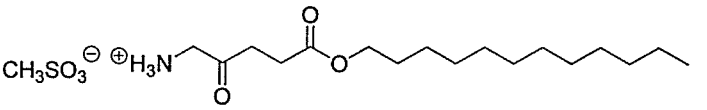
C10-S (decyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 0.854 (t, 3H, CH_3), 1.244-1.281 (m, 14H, $7CH_2$), 1.537 (m, 2H, CH_2), 2.317 (s, 3H, CH_3), 2.546 (t, 2H, CH_2), 2.786 (t, 2H, CH_2), 3.972 (s, 2H, CH_2), 3.989 (t, 2H, CH_2), 8.042 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 13.99, 22.14, 25.38, 27.10, 28.12, 28.72, 28.74, 28.99, 31.34, 34.23, 39.64, 46.77, 64.13, 172.11, 202.75.

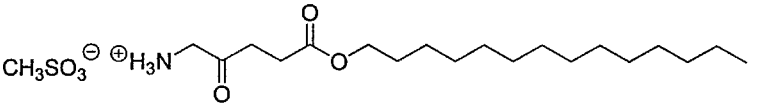
C12-S (dodecyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 0.854 (t, 3H, CH_3), 1.242~1.285 (m, 18H, $9CH_2$), 1.550 (m, 2H, CH_2), 2.317 (s, 3H, CH_3), 2.555 (t, 2H, CH_2), 2.786 (t, 2H, CH_2), 3.968 (s, 2H, CH_2), 3.991 (t, 2H, CH_2), 8.029 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 13.95, 22.09, 28.70, 28.97, 29.00, 29.03, 31.28, 39.64, 46.72, 64.11, 172.07, 202.77.

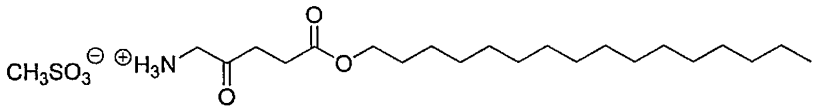
C14-S (tetradecyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 0.853 (t, 3H, CH_3), 1.238-1.280 (m, 22H, $11CH_2$), 1.549 (m, 2H, CH_2), 2.312 (s, 3H, CH_3), 2.549 (t, 2H, CH_2), 2.786 (t, 2H, CH_2), 3.968 (s, 2H, CH_2), 3.990 (t, 2H, CH_2), 8.025 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 13.95, 22.09, 28.09, 28.67, 28.70, 28.94, 29.03, 31.29, 39.64, 46.72, 64.11, 172.07, 202.76.

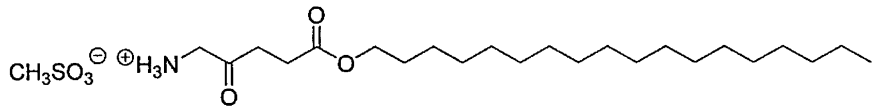
C16-S (hexadecyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 0.854 (br. s, 3H, CH_3), 1.238 (br. s, 26H, 13CH_2), 1.550 (br. s, 2H, CH_2), 2.329 (br. s, 3H, CH_3), 2.549 (br. s, 2H, CH_2), 2.786 (br. s, 2H, CH_2), 3.976 (br. s, 4H, 2CH_2), 8.027 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 13.90, 22.04, 25.30, 28.05, 28.64, 28.98, 31.24, 34.19, 39.64, 46.70, 64.08, 172.01, 202.69.

C18-S (octadecyl 5-aminolevulinate methanesulfonate)



^{1}H NMR (DMSO) δ : 0.856 (br. s, 3H, CH_3), 1.239 (br. s, 30H, 15CH_2), 1.551 (br. s, 2H, CH_2), 2.307 (br. s, 3H, CH_3), 2.554 (br. s, 2H, CH_2), 2.786 (br. s, 2H, CH_2), 3.974 (br. s, 4H, 2CH_2), 8.010 (br. s, 3H, NH_3).

^{13}C NMR (DMSO) δ : 13.89, 22.03, 27.04, 28.05, 28.63, 28.96, 31.23, 34.19, 39.36, 46.72, 64.07, 172.01, 202.71.

Elemental analyses are shown in Table 1. Definitely, the measured results are very close to the calculated values. The above results confirm the chemical structure of as-prepared ALA and its alkylester methanesulfonates, i.e. $CH_3SO_3H \cdot H_2NCH_2COCH_2$ -CH_2COOR (R=H, CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_8H_{17}, $C_{10}H_{21}$, $C_{12}H_{25}$, $C_{14}H_{29}$, $C_{16}H_{33}$, $C_{18}H_{37}$), labeled as C0-S, C1-S, C2-S, C3-S, C4-S, C5-S, C6-S, C8-S, C10-S, C12-S, C14-S, C16-S, C18-S, respectively.

3.2 Lipophilicity

The lipophilicity of methanesulfonates of both ALA and its alkylesters was assessed by measuring the apparent partition coefficient (P) of the compounds between octanol and a PBS solution of pH 7.4. Table 2 summarizes the obtained log *P* values. The results plotted in Fig. 2 show that it is possible to vary the lipophilicity of ALA compounds by more than three orders of magnitude when using ALA esters. The log *P* values of C0-S (ALA), C1-S(m-ALA), C2-S(e-ALA) and C3-S(pr-ALA) are negative, representing the hydrophilic feature of these substances. But their hydrophilic properties decrease with carbon number of alkylester from 3 to 0. Relative to ALA and C1-S(m-ALA), C2-S(e-ALA) and C3-S(pr-ALA), all other esters are more lipophilic with positive log *P* values. When carbon number of alkylester is more than 5, such as $C_{6\text{-}16}$-S, the log *P* tends to reach a constant value of 2.3-2.4. It should be mentioned that C18-S shows a high log *P* of 3.04, which implies the most lipophilic in these samples. Definitely, the above results are similar to that of the ALA ester hydrochlorides reported before [18].

Table 1 Elemental analyses results

Sample	C (wt%)	H (wt%)	N (wt%)	S (wt%)
C0-S	31.85 (31.72)	5.64 (5.77)	6.10 (6.16)	14.35 (14.11)
C1-S	34.67 (34.85)	5.94 (6.27)	5.71 (5.81)	13.41 (13.29)
C2-S	37.47 (37.64)	6.21 (6.71)	5.34 (5.49)	12.72 (12.56)
C3-S	40.01 (40.14)	6.86 (7.11)	5.06 (5.20)	12.01 (11.91)
C4-S	42.17 (42.39)	7.01 (7.47)	4.76 (4.94)	11.42 (11.32)
C5-S	44.21 (44.43)	7.32 (7.80)	4.63 (4.71)	10.91 (10.78)
C6-S	45.98 (46.28)	7.63 (8.09)	4.29 (4.50)	10.41 (10.30)
C8-S	48.19 (49.54)	8.41 (8.61)	4.04 (4.13)	9.86 (9.45)
C10-S	51.49 (52.29)	8.71 (9.05)	3.63 (3.81)	8.96 (8.73)
C12-S	52.09 (54.86)	9.15 (9.43)	3.43 (3.54)	8.64 (8.11)
C14-S	55.22 (56.71)	9.40 (9.76)	3.18 (3.31)	7.78 (7.57)
C16-S	56.86 (58.50)	9.61 (10.04)	2.96 (3.10)	7.36 (7.10)
C18-S	58.51 (60.09)	9.84 (10.30)	2.80 (2.92)	6.90 (6.68)

* Data in ( ) represents a calculated value from chemical formula.

Table 2 Apparent partition coefficient of ALA derivatives

Sample	Log *P*
C0-S	-1.48 ± 0.13
C1-S	-1.03 ± 0.10
C2-S	-0.61 ± 0.06
C3-S	-0.13 ± 0.02
C4-S	0.79 ± 0.05
C5-S	1.54 ± 0. 11
C6-S	2.26 ± 0.12
C8-S	2.34 ± 0.16
C10-S	2.41 ± 0.17
C12-S	2.41 ± 0.18
C14-S	2.40 ± 0.16
C16-S	2.50 ± 0.17
C18-S	3.04 ± 0.19

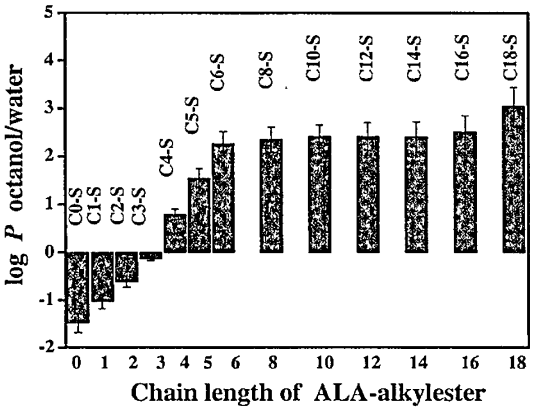


Fig. 2 Log P versus carbon number of ALA derivatives

3. Conclusion

NMR and elemental analysis techniques confirm that a series of ALA alkylester methanefulfonates were synthesized successfully through a reaction of ALA hydrochloride with alkanol. ALA and low carbon ($C_{1\text{-}3}$-S) methanesulfonates show the hydrophilic feature meanwhile $C_{(4\text{-}18}$-S) exhibit more lipophilic character with carbon number of alkylester. When carbon number is more than 5, Log*P* almost reaches a constant value of 2.3-2.4.

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