New Process for the Exclusive γ-Conjugation of Folic Acid for Targeted Drug Delivery

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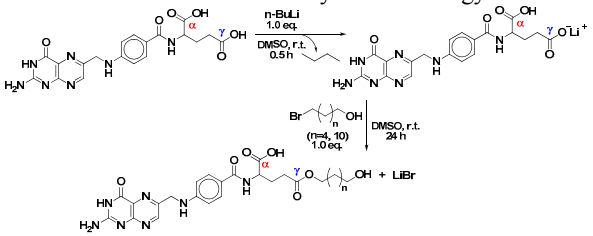
Statement of Purpose: Selectively delivering drugs to inflammatory sites, thereby avoiding the collateral damage of healthy cells is a very important objective[1]. Folic acid (FA) has emerged as an important targeting ligand for the selective delivery of attached therapeutic and imaging agents, because FA targets cancer tissues and sites of inflammation that overexpress folate receptor (FR) proteins bounded on the cell membrane[2]. The fact that high affinity FR binding is retained when FA is covalently linked via its γ-carboxylate of the glutamic acid moiety to a cancer drug makes it a good choice for targeted drug delivery[3]. In most cases the conjugation of FA with bioactive agents is carried out by the so-called "activated ester" method using condensing agents[4]. However, the regioselectivity toward the desired γ-conjugated product is quite poor, because of the very similar reactivity of the two carboxylic acid groups (α and γ) of FA. Exclusive γ-conjugation was achieved by a "retrosynthetic" approach, based on the cleavage of the FA into pteroate and glutamate derivatives, followed by selective protection and reconstruction of the γ-folate[5,6], involving multiple steps with not very high efficiency. In this paper, we describe a new process for the exclusive γ-conjugation of folic acid with high selectivity and efficiency[7].

Methods: Folic acid (FA, Sigma), n-butyllithium (n-BuLi, 99 %, Aldrich), 6-bromo-1-hexanol, 12-bromo-1-dodecanol (TCI America), dimethyl sulfoxide (DMSO, anhydrous, 99.9 %, Sigma), tetrahydrofuran (THF), diethyl ether, and hexane (Fisher Scientific) were used as received. Scheme 1 shows the synthetic strategy.



Scheme 1. Synthetic strategy for γ-conjugation of FA.

2.000 g of FA (4.531 mmol, 0.1 M) was dissolved in 45.2 mL of anhydrous DMSO overnight under argon gas. 2.265 mL of *n*-BuLi (4.531 mmol, 1 eq.) was added dropwise into the FA solution using a dropping funnel. The solution was stirred for 30 min at 20°C. Subsequently 1.296 g of 12-Bromo-1-dodecanol (4.531 mmol, 1 eq.) was added into the reaction mixture. The solution was stirred for 24 hrs at room temperature. The dark yellow product was precipitated in 400 mL diethyl ether and washed with hexane and THF. Finally the brownish product was intensively washed with water to remove free FA and LiBr. ^{1}H and ^{13}C NMR spectra were recorded on a Varian NMRS 500 spectrometer in deuterated dimethyl sulfoxide (DMSO-d_6). The DMSO-d_6 peak was used as an internal reference (^{1}H NMR, δ = 2.50 ppm; ^{13}C NMR, δ = 39.51 ppm).

Results: The ^{1}H NMR spectrum of FA-dodecanol in DMSO-d_6 is shown in Figure 1. There is no proton resonance signal at δ=3.45 ppm, characteristic of the CH_2 protons next to the -Br group in 12-bromo-1-dodencanol. The CH_2 protons (*x*) of the dodecanol conjugated to the γ-carboxylic acid in FA appear at 4.00 ppm, indicating complete reaction. Also, the –NH proton (*r*) doublet at δ=8.11-8.12 ppm in the free FA shifted to a new doublet signal (*r'*) of the γ-substituted product at δ=8.23-8.24 ppm. In addition, there were no signals at δ=7.80-8.00 ppm, indicating no α-conjugation or α/γ-mixtures.

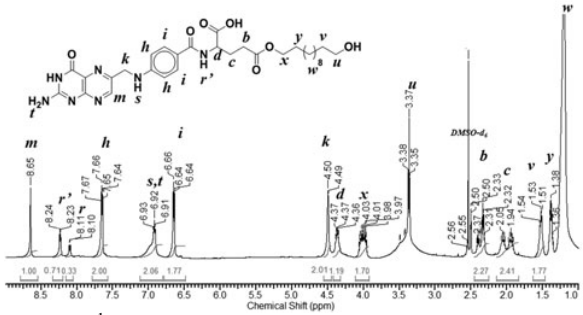


Figure 1. ^{1}H NMR of FA-dodecanol in DMSO-d_6

The same procedure was repeated with 6-Br-1-hexanol, yielding also exclusive γ-conjugation.

Conclusions: We successfully developed an efficient synthetic process for the exclusive γ-conjugation of the glutamic acid moiety of FA by the sequential processes of the lithiation of FA with n-BuLi, followed by the reaction of FA-γLi with 1-bromo-alcohols, producing γ-substituted FA with an –OH group available for further conjugation with other bioactive molecules. This method has a general applicability in targeted drug delivery.

References:

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