

## A SIMPLE AND EFFICIENT SYNTHESIS OF 3-2 PYRIDINYLDITHIO PROPANOIC ACID HYDRAZIDE: A HETEROBIFUNCTIONAL CROSSLINKER

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Received: 02 Jun 2012, Revised and Accepted: 12 July 2012

### ABSTRACT

3-2 Pyridinyldithio propanoic acid hydrazide (PDPH) is a very important heterobifunctional crosslinker that is widely used in bioconjugate techniques for making macromolecule-drug conjugates. In the present study, we report a simple and efficient procedure for the synthesis of PDPH in good yield by coupling 3,2 pyridinyldithio propanoic acid with *tert*-butyl carbazate and 1-ethyl 3-(3-dimethylamino propyl) carbodiimide hydrochloride.

**Keywords:** PDPH, Heterobifunctional crosslinker, Synthesis of PDPH, Coupling reaction

### INTRODUCTION

Bifunctional cross-linking reagents are extremely important compounds in bioconjugate chemistry. They are widely used to introduce various reactive functional groups such as maleimido groups<sup>1</sup>, sulfhydryl group<sup>2</sup>, 2-pyridyldithio group<sup>3-5</sup>, and azido groups<sup>6</sup>, for making macromolecule-drug conjugates<sup>7-8</sup>. These reagents are used as a spacer or cross-bridge that ties both the drug and macromolecule together. 3-2 Pyridinyldithio propanoic acid hydrazide (PDPH) is a heterobifunctional crosslinker containing two different reactive groups that can couple to two different functional targets. One part of the crosslinker contains an amine-reactive group that reacts with a drug molecule, while the other contains a sulfhydryl-reactive group that reacts with a macromolecule in a two- or three-step process<sup>9</sup>. PDPH is a very useful for a number of biological applications<sup>10-15</sup>. It is not readily available in bulk quantity. This is apparently due to the absence of effective synthetic routes to make this chemical<sup>16</sup>.

Doxorubicin is widely used as an anti-tumor agent for solid tumors. Its use, however, is dose limited due to its cardiac and other toxicities. In an attempt to minimize these toxicities, it was proposed by the authors to conjugate Doxorubicin to thermosensitive polymers, such as Elatin Like Polypeptides (ELPs), with the objective of targeting the drug to solid tumors. This requires the use of the bifunctional cross linking agent, namely PDPH. We started synthesizing PDPH, as per the procedure given by Kaneko et. al.<sup>17-20</sup>, but we faced a problem in getting the crosslinker in good yield. This paper reports on the problem we faced and how we were able to overcome the problem by modifying the procedure adopted by Kaneko, to obtain PDPH in good yield.

### MATERIALS AND METHODS

All solvents were ACS ~~certi~~ grade purchased from Fisher Scientific/Sigma and used without further purification unless otherwise noted. Flash chromatography was performed with indicated solvent system using 200-400 mesh silica gel. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded at 400 MHz (Bruker Avance 400). Chemical shifts were reported in  $\delta$  value. Mass spectra were from Micromass Q-TOF micromass using electron spray ionization mode. Experiments were conducted at ambient temperature, unless otherwise stated.

### RESULTS AND DISCUSSION

Methoxy carbonyl sulfonyl chloride (0.001 mole, 1 equiv) was dissolved in 20 ml of dichloro methane (DCM). A solution of 3 mercaptopropionic acid (0.001 mole, 1 equiv) in 20 ml of DCM was then added to the solution of methoxy carbonyl sulfonyl chloride in DCM dropwise for 3h. As the reaction is sensitive to light or moisture the reaction vessel was purged with nitrogen, capped, and stirred for 24 h at room temperature taking all the required

precautions and concentrated under vacuum. The residue obtained was redissolved in DCM and added dropwise to a solution of 2 mercaptopyridine (0.001 mole, 1 equiv). After stirring overnight, the solvent was evaporated and the oily product 3-2 pyridinyl di thio propanoic acid (**1**) (yield 70-80 %) was isolated.

The <sup>1</sup>H NMR spectrum of (**1**) (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.49 (1H, s, Py),  $\delta$  = 7.65 (1H, s, Py),  $\delta$  = 7.67- 7.14 (3H, m, Py), 3.07 (2H, t, J = 12.8 Hz, CH<sub>2</sub>S), 2.85 (2H, t, J = 12.8 Hz, CH<sub>2</sub>CO). <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>),  $\delta$  = 177.1 (s), 159.1 (s), 149.3 (s), 137.5 (s), 121.2 (d), 34.0 (s), 33.8 (s). ESI m/z = 237.99 [M + Na]<sup>+</sup> (calculated molecular weight 215.29 for chemical formula C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>S<sub>2</sub>).

Product (**1**) was dissolved in anhydrous THF, stirred and chilled in ice, and treated in succession with TEA, isobutyl chloroformate and 5 min later, *tert*-butyl carbazate. The reaction was stirred at room temperature for 1 h and then partitioned between DCM and water. The organic phase was washed and dried and the solvent evaporated. The residue was dissolved in ice cold TFA and the solution stirred at 0°C for 10 min and additional 10 min at room temperature. The excess TFA was evaporated and the residue was chromatographed on silica gel using DCM: MeOH: NH<sub>4</sub>OH (50:2.5:0.25) solvent system.

At this point we faced a problem in deprotecting the Boc group from Boc 3-2 pyridinyldithio propanoic acid hydrazide to get pure PDPH. A TFA treatment gives a mixture for Boc products, namely, Boc 3-2 pyridinyldithio propanoic acid hydrazide and 3-2 pyridinyldithio propanoic acid hydrazide. No complete cleavage of Boc occurs. The yield we obtained was less than 30% and not 50% as claimed by Kaneko et. al. It may be pointed out here that the theoretical yield as per Kaneko's procedure is only 35% which is almost the yield we obtained using Kaneko's procedure. Scheme 1 shows the preparation of PDPH as per Kaneko's procedure.

### Reagents and conditions

i) DCM, 3h, r.t. ii) 2-mercaptopyridine, overnight, r.t. iii) isobutylchloroformate/TEA 10 min, 0° C iv) *tert*-butyl carbazate 1h, r.t. v) TFA 10 min, 0°C - r.t.

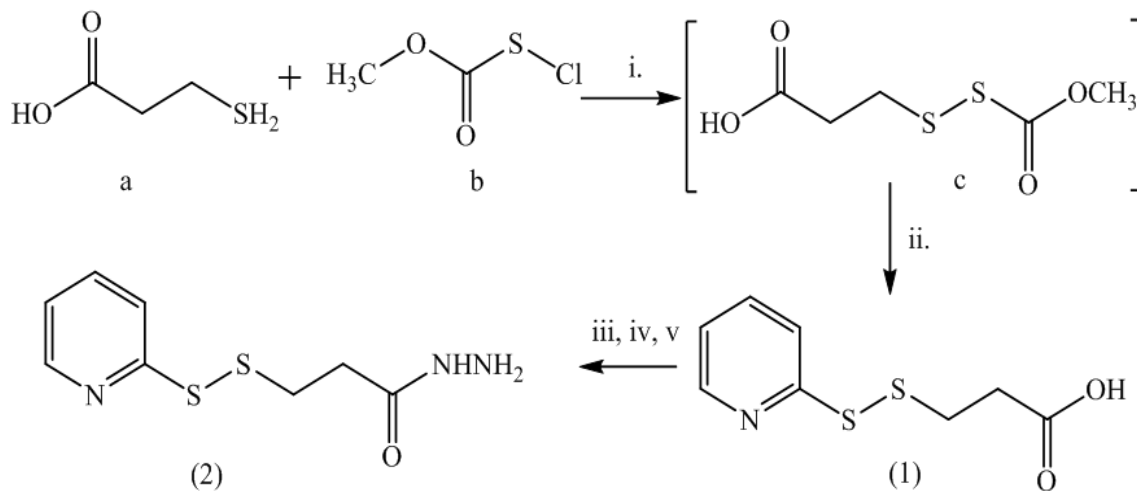
a) 3-mercapto propionic acid b) methoxy carbonyl sulfonyl chloride c) an unstable intermediate formed

We adopted a different procedure to improve the yield of PDPH. Product (**1**) (100 mg, 0.465 mmol) was dissolved in the acetonitrile in an inert atmosphere. HOBt (221.2 mg, 1.86 mmol), *tert*-butyl carbazate (57 mg, 0.93 mmol), DIEA (180 mg, 1.395 mmol) dried over molecular sieves and 1-ethyl 3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDC.HCl 118 mg, 0.93 mmol) were added to the above and stirred for 5 h, first at 0°C and then at room temperature maintaining anhydrous conditions. After the completion of the reaction, the residue was dissolved in water and extracted with organic solvent. The solution was concentrated under

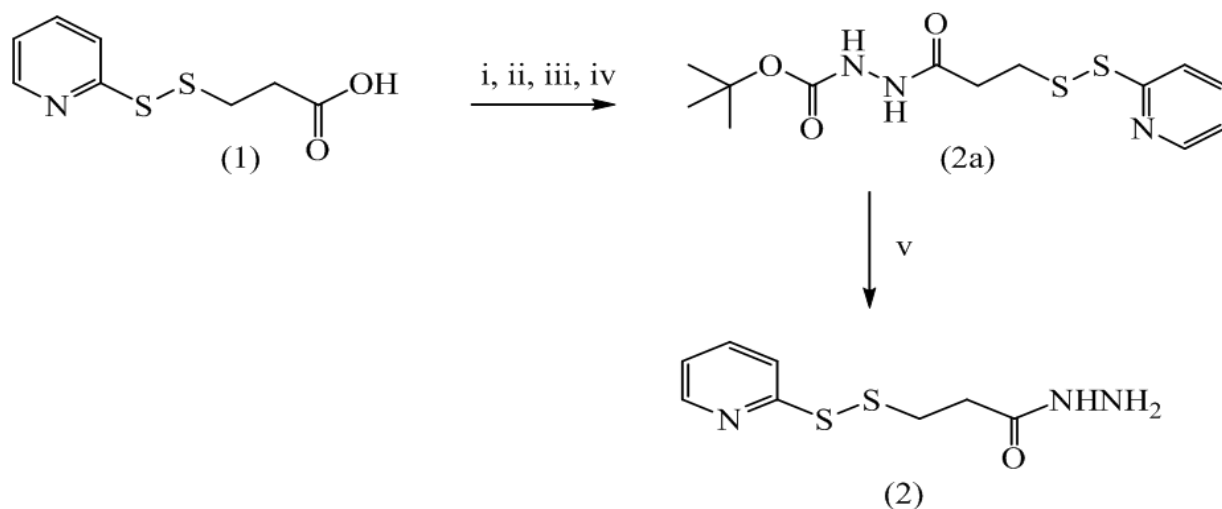
vacuum to obtain Boc 3-2 pyridinyl dithio propionic acid hydrazide (**2a**).

To a stirred solution of **2a** (100 mg, 0.303 mM) in 10 ml of DCM cooled to 0°C, trimethylsilyl trifluoromethanesulfonate (101 mg, 0.4559 mM) was then added. It was stirred at the same temperature for 10 min. DCM layer was decanted. A gummy solid 3-2 pyridinyl dithio propanoic acid hydrazide (**2**) was obtained (yield 55-55 %).

The residue was chromatographed on silica gel using DCM: MeOH: NH<sub>4</sub>OH (50:2.5:0.25) solvent system. Product (**2**) obtained was further purified by crystallization from ethyl acetate to yield a crystalline residue (yield 50-55%). It was characterized by <sup>1</sup>H, <sup>13</sup>C NMR. Spectral and analytical data of the synthesized PDPH were all in good agreement with the proposed structure. Scheme 2 represents synthetic route for the preparation of PDPH adopted by us.



Scheme 1



Scheme 2

#### Reagents and conditions

i) EDC.HCl, CH<sub>3</sub>CN, ii) DIEA, iii) HOBt, iv) tert-butyl carbazate, 5h, 0°C- r.t., **2a**) Boc 3-2 pyridinyl dithio propanoic acid hydrazide, v) trimethylsilyl trifluoromethane sulfonate, 10 min, 0°C- r.t.

The <sup>1</sup>H NMR spectrum of PDPH (400 MHz, CDCl<sub>3</sub>) δ = 10.98 (1H, s, NH), δ = 10.12-10.54 (2H, s, NH<sub>2</sub>), δ=8.49- 7.14 (4H, m, Py), 2.80 (2H, t, J = 6.62Hz, CH<sub>2</sub>S), 3.06 (2H, t, J = 6.45 Hz, CH<sub>2</sub>N). <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>), δ = 158.5 (d), 149.6 (s), 129.1 (s), 121.3 (s), 120.4 (s), 55.6 (s), 34.0 (s). ESI m/z = 252 [M + Na]<sup>+</sup> (calculated molecular weight 229 for chemical formula C<sub>8</sub>H<sub>11</sub>N<sub>3</sub>OS<sub>2</sub>).

#### CONCLUSION

In conclusion, we report a simple and highly efficient procedure for the synthesis of 3-2 pyridinyl dithio propanoic acid hydrazide (PDPH) from 3-2 pyridinyl dithio propanoic acid with improved

yields (50-55%). PDPH prepared will be used by us for the preparation of some polymeric pro-drugs for cancer chemotherapy. Work in this direction is in progress.

#### ACKNOWLEDGEMENT

Authors acknowledge Dr. R. Balamurali and Dr. S. Chandrasekaran for helpful suggestions. We are grateful to the Department of Science & Technology, Government of India., for funding, under Women Scientist Scheme.

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