Supporting Information

Novel Phosphate Modified Cathepsin B Linkers: Improving Aqueous Solubility and Enhancing Payload Scope of ADCs

Authors: Jeffrey C. Kern[1*], Deborah Dooney[2], Rena Zhang[2], Linda Liang[3], Philip E. Brandish[4], Mangeng Cheng[5], Guo Feng[5], Andrew Beck[6], Damien Bresson[6], Juhi Firdos[6], Dennis Gately[6], Nick Knudsen[6], Anthony Manibusan[6], Ying Sun,[6] Robert M. Garbaccio[1]

Author Affiliations:
[1]Global Chemistry, Merck & Co., Inc., 770 Sumneytown Pike, West Point, Pennsylvania 19486, United States;
[2]PPDM, Merck & Co., Inc., 770 Sumneytown Pike, West Point, Pennsylvania 19486, United States;
[3]Biologics Merck & Co., Inc., 901 California Avenue, Palo Alto, California 94304, United States;
[4]Biology, Respiratory and Immunology, Merck & Co., Inc., 33 Avenue Louis Pasteur, Boston, Massachusetts 02115, United States;
[5]In Vitro Pharmacology, Merck & Co., Inc., 33 Avenue Louis Pasteur, Massachusetts 02115, United States ;
[6]Ambrx, 10975 North Torrey Pines Road, San Diego, California 92121, United States.

*Email: Jeffrey.Kern@Merck.com

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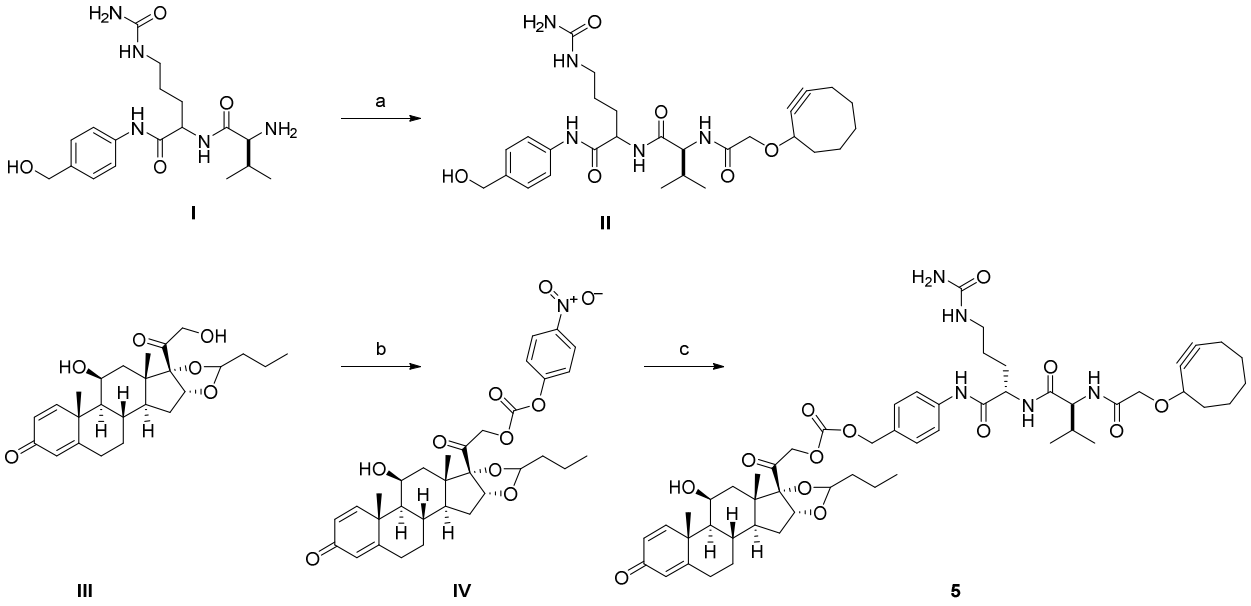
Experimental Procedures:



a.2-(cyclooct-2-yn-1-yloxy)acetic acid, HATU, DMF, rt, 43%. b. 4-nitrophenylchloroformate, Et_3N, DCM, rt, 59% c. **II**, DMAP, DCM, rt, 18%.

Scheme A: Synthesis of Budesonide Carbonate Cathepsin B cleavable linker molecule **5.**

Step A : *2-((2S)-2-(2-(cyclooct-2-yn-1-yloxy)acetamido)-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide* (**II**)

To a stirred solution of **I** (0.20 g, 0.53 mmol) and 2-(cyclooct-2-yn-1-yloxy)acetic acid (0.10 g, 0.58 mmol) in DMF (1.2 mL) was added HATU (0.20 g, 0.53 mmol) and the resulting solution was stirred 10 minutes at room temperature. The reaction directly purified using reverse phase preparative chromatography (Sunfire Prep C18 OBD 5 um 30 x 150 mm; 20-70%

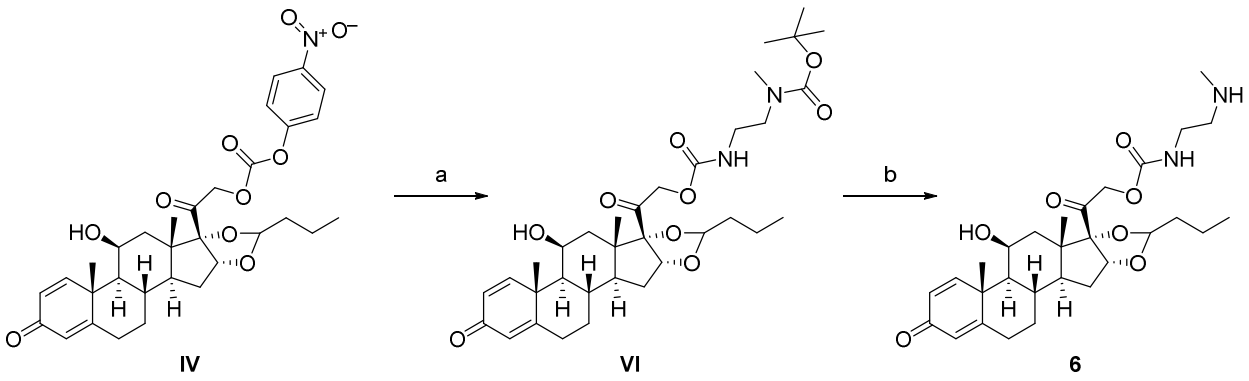
CH3CN/water w/ 0.1% TFA modifier over 20 min) to give **II** as a solid (123 mg, 43%). LRMS (ES) (M+H)+ : observed = 544.5, calculated = 543.6.

Step B : *2-((6aR,6bS,7S,8aS,8bS,11aR,12aS,12bS)-7-hydroxy-6a,8a-dimethyl-4-oxo-10-propyl-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-8b-yl)-2-oxoethyl (4-nitrophenyl) carbonate* (**IV**)

To a stirred solution of budesonide (**III**, 0.30 g, 0.70 mmol) and triethylamine (0.29 mL, 2.10 mmol) in DCM (4.0 mL) was added 4-nitrophenyl chloroformate (0.28 g, 1.39 mmol) and the resulting solution was stirred 5 minutes at room temperature. The reaction directly purified by flash column separation using a 0-50% ethyl acetate/ hexane gradient to give **IV** as a solid (245 mg, 59%). LRMS (ES) (M+H)+ : observed = 596.5, calculated = 595.6.

Step C : *4-((2S)-2-((2S)-2-(2-(cyclooct-2-yn-1-yloxy)acetamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl (2-((6aR,6bS,7S,8aS,8bS,11aR,12aS,12bS)-7-hydroxy-6a,8a-dimethyl-4-oxo-10-propyl-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-8b-yl)-2-oxoethyl) carbonate* (**5**)

To a stirred solution of **IV** (0.05 g, 0.08 mmol) and **II** (0.046 g, 0.08 mmol) in DCM (0.5 mL) and DMF (0.5 mL) was added DMAP (0.01 g, 0.08 mmol) and the resulting solution was stirred at room temperature. Additional **IV** was added portionwise until **II** was consumed. The reaction conc to remove DCM, diluted with DMF and directly purified using reverse phase preparative chromatography (Sunfire Prep C18 OBD 5 um 30 x 150 mm; 20-90% CH3CN/water w/ 0.1% TFA modifier over 20 min) to give **5** as a solid (15 mg, 18%). HRMS calcd for $C_{54}H_{73}N_5O_{13}$ $(M+H)^+$ 1000.5283, found 1000.5296



a. tert-butyl (2-aminoethyl)(methyl)carbamate, DCM, rt, 77%. b. TFA, DCM, rt, 96%

Scheme B: Synthesis of Budesonide Carbamate molecule **6.**

Step A : *tert-butyl (2-((((2-((6aR,6bS,7S,8aS,8bS,11aR,12aS,12bS)-7-hydroxy-6a,8a-dimethyl-4-oxo-10-propyl-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-*

naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-8b-yl)-2-oxoethoxy)carbonyl)amino)ethyl)(methyl)carbamate (**VI**)

To a stirred solution of **IV** (0.10 g, 0.168 mmol) in DCM (1.0 mL) was added tert-butyl (2-aminoethyl)(methyl)carbamate (0.058 g, 0.33 mmol) and the resulting solution was stirred 20 minutes at room temperature. The reaction directly purified by flash column separation using a 0-100% ethyl acetate/ hexane gradient to give **VI** as a solid (82 mg, 77%). LRMS (ES) (M+H)+ : observed = 631.4, calculated = 630.7.

Step B : *2-((6aR,6bS,7S,8aS,8bS,11aR,12aS,12bS)-7-hydroxy-6a,8a-dimethyl-4-oxo-10-propyl-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-8b-yl)-2-oxoethyl (2-(methylamino)ethyl)carbamate* (**6**)

To a stirred solution of **VI** (0.082 g, 0.13 mmol) in DCM (1.0 mL) was added TFA (1.0 mL, 12.89 mmol) and the resulting solution was stirred 35 minutes at room temperature. The reaction was concentrated, dissolved in ethyl acetate and washed several times with saturated sodium bicarbonate solution. The organic phase was dried over sodium sulfate to give **6** as a solid (51 mg, 96%). LRMS (ES) (M+H)+ : observed = 531.6, calculated = 530.6.

Stability tests of Budesonide Carbamate 6

In two vials was dissolved 5mg budesonide carbamate **6** in DMSO (282 ul). To one vial was added PBS buffer pH 7.4 (141 ul), to the other vial was added PBS buffer pH 5.0 (141 ul) to give a final solution concentration of 0.022 M. Both solutions were heated to 37°C for 24 hrs. Stability was monitored by LCMS at time = 0 and 24 hr. In both solutions parent budesonide carbamate **6** remained intact, no free budesonide was observed.

Human Blood stability screening of 5, 8a, 8b

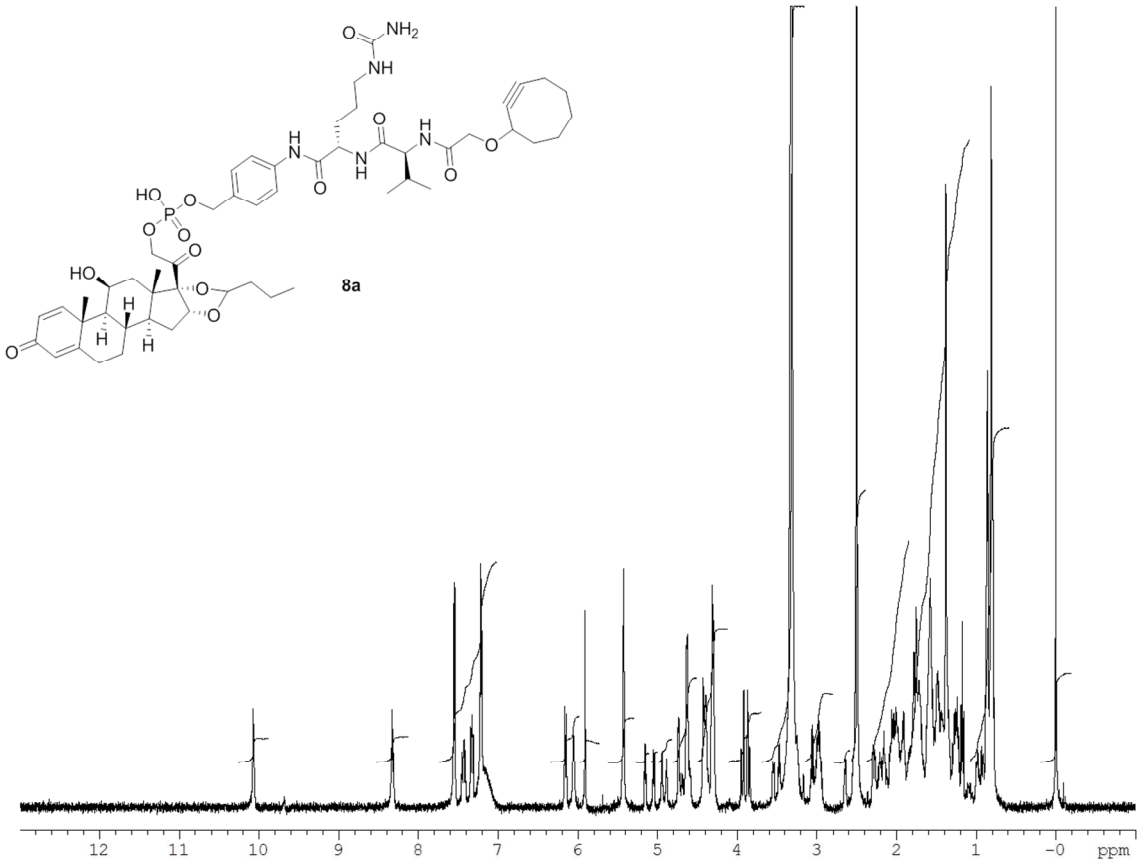
Table-1 Time Course of Calculated Conc. for each compound (nM)

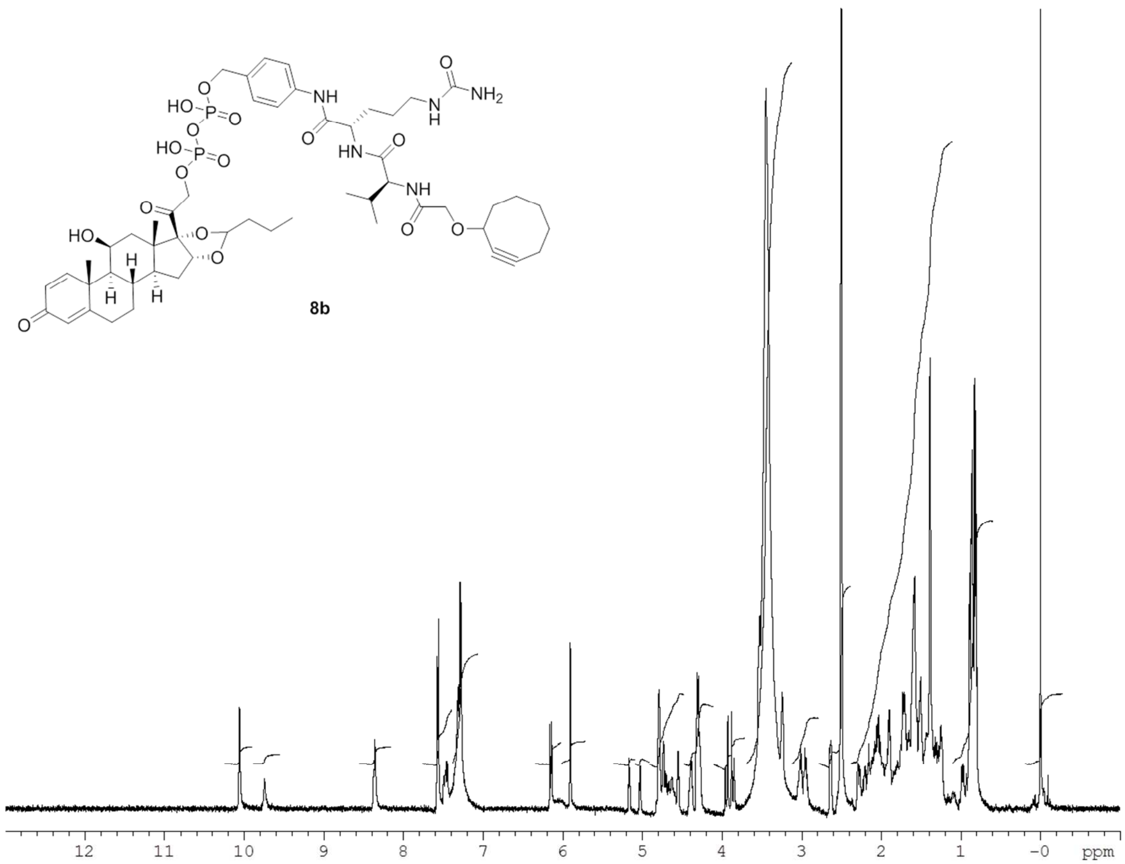
	Budesonide	8a	8b	5
Time	Human Blood	Human Blood	Human Blood	Human Blood
Matrix spiking	3893.06	5641.52	5077.24	2410.67
0m	1354.74	1629.88	1805.21	864.86
20m	1306.68	2117.92	1925.77	893.7
1hr	1409.64	1644.76	1906.17	758.12
3hr	1378.66	1648.37	1879.85	668.34
6hr	1245.05	1994	1683.4	594.83

Table-2 Time Course of Budesonide Calculated Conc. for each compound (nM)

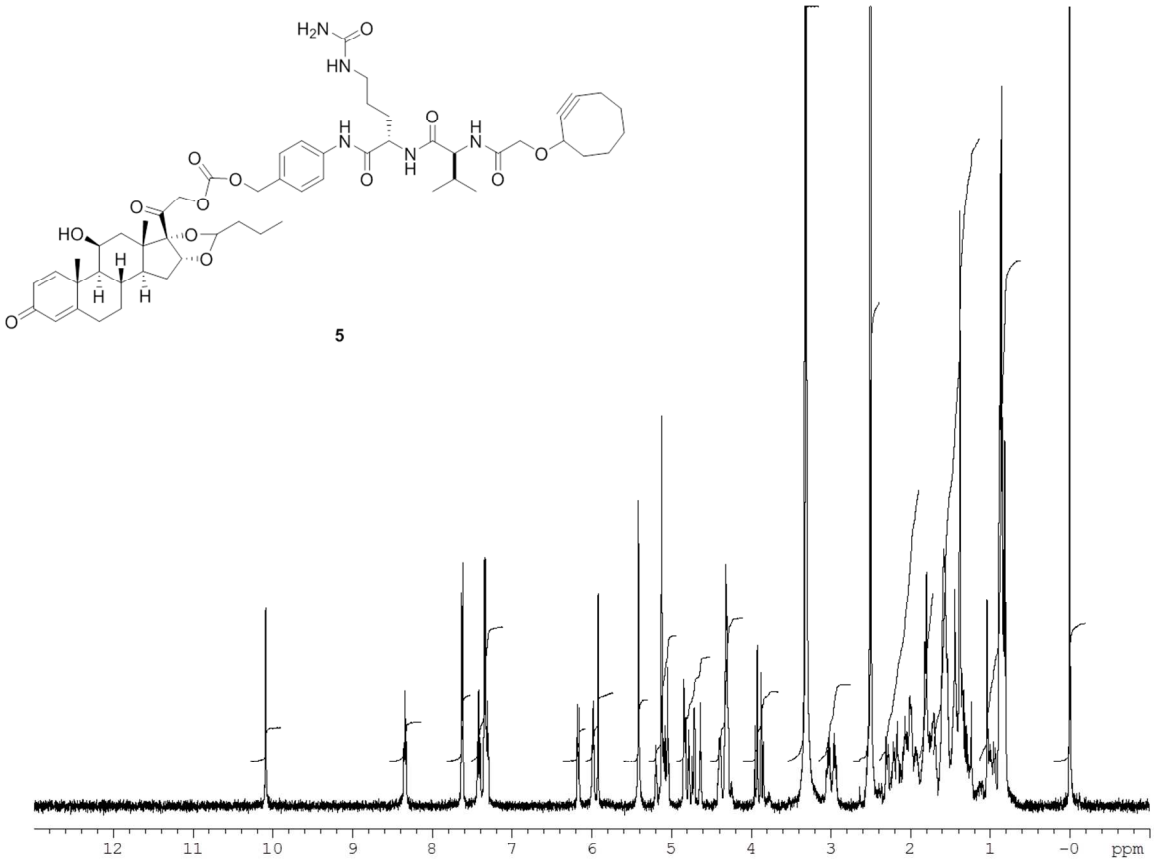
Time	Human Blood	Human Blood	Human Blood	Human Blood
Matrix spiking	3893.06	< 0	< 0	61.21
0m	1354.74	< 0	< 0	< 0
20m	1306.68	< 0	< 0	13.54
1hr	1409.64	< 0	8.48	7.56
3hr	1378.66	< 0	8.17	26.29
6hr	1245.05	4.1	1.9	37.72

NMR data


8a
12
11
10
9
8
7
6
5
4
3
2
1
-0
ppm



8b



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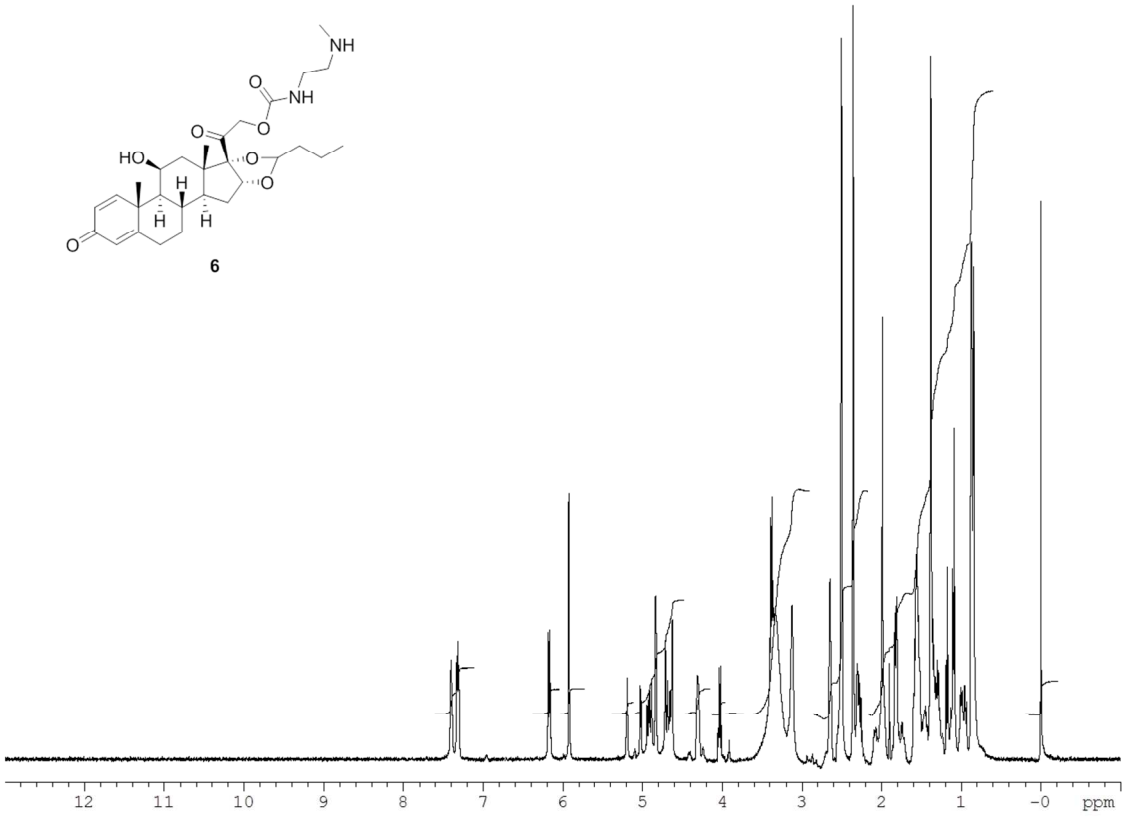

6

End