

## 2-(*p*-Hydroxybenzyl)indoles - Side Products Formed Upon Cleavage of Indole Derivatives from Carboxylated Wang Polymer - an NMR Study

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**Abstract:** Treatment of carboxylated Wang polymer attached to a 2-unsubstituted indole derivative with a trifluoroacetic acid based mixture resulted in a side reaction: *p*-hydroxybenzylation at the 2-position of the indole ring. The structure of the resulting N-3-aminopropyl)-N-benzyl-4-[2-(4-hydroxybenzyl)-1H-indol-3-yl]-butyramide trifluoroacetate was ascertained by a full assignment of its <sup>1</sup>H- and <sup>13</sup>C-NMR spectra. The side reaction could be suppressed by the use of 1,2-ethanedithiol in high concentrations (16 %).

**Keywords:** Carboxylated Wang polymer; side reaction; *p*-hydroxybenzylation; 1,2-ethanedithiol; NMR.

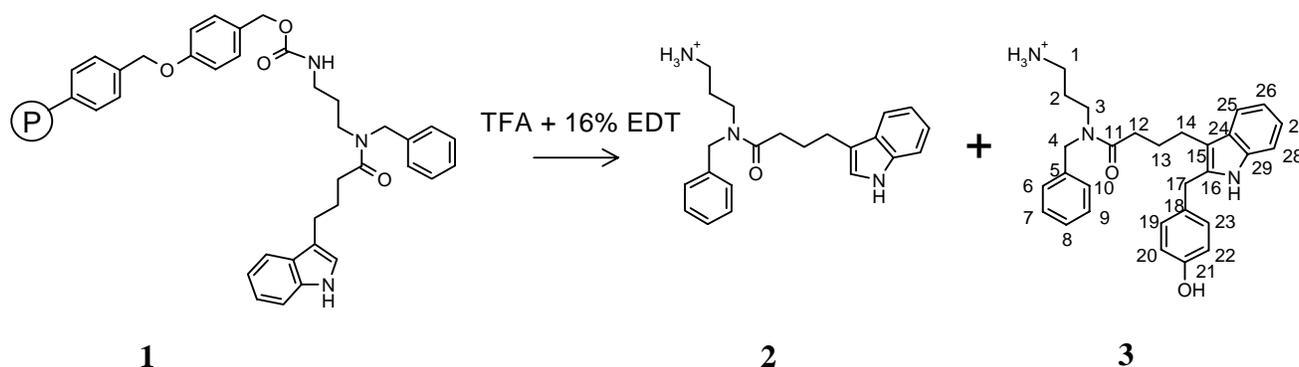
## Introduction

Treatment with trifluoroacetic acid plus additives is a standard procedure to cleave off reaction products from Wang polymers. However, when using this method in solid phase synthesis of some tryptophan residue containing peptides alkylation of an indole ring present was observed as a side reaction [1,2]. This substitution was earlier reported to mainly affect position 2, but it is also known to touch position 3 and the benzene ring of the indole moiety in some cases [1]. Moreover, strong dependence on the investigated structure was reported; as an example, the tryptophan residue at the C-terminus of a peptide did not form any side products under the same conditions [1,2]. Unfortunately, in the early reports mentioned above [1,2] no detailed NMR data were given.

## Results and Discussion

In the course of our studies on the synthesis of tertiary amides (i.e., compound **2**), formation of similar side products (compound **3**) was observed (for an example see Scheme 1).

Scheme 1



Carboxylated Wang polymers attached to tertiary amides containing an indole moiety (compound **1** in the representative example) were treated with a trifluoroacetic acid based cleavage cocktail. LC/MS analysis of the cleaved products showed the presence of a substance **3** having a molecular ion with a mass exceeding the desired one by 106 daltons and corresponding to alkylation of the tertiary amide by the *p*-hydroxybenzyl group, possibly coming from the spacer of the Wang polymer. These side products **3** were isolated as trifluoroacetates using reversed phase preparative HPLC, where they displayed retention times slightly longer than those of the tertiary amides **2**. During TLC analysis

(Merck Silica Gel 60 plates, eluent: 1-butanol-acetic acid –water, 4:1:1) the side products gave violet-grey spots (1 % FeCl<sub>3</sub> in ethanol spray and keeping overnight at room temperature), a colour indicating the presence of the phenolic hydroxyl group [3]. This was never observed on spots of the tertiary amides **2**.

The obtained yield of these *p*-hydroxybenzylated products was 10 to 30% of the main product yield, even in the presence of scavengers. In control experiments side product formation could be considerably reduced by the use of 1,2-ethanedithiol as an additive. Consequently, we routinely used a 40:8:1:1 trifluoroacetic acid-1,2-ethanedithiol-triisopropylsilane-water mixture for the preparation of tertiary amides such as **2**. In an attempt to increase the yield of the side products, the polymer attached tertiary amide **1** was cleaved with trifluoroacetic acid without any additives. In this case, the *p*-hydroxybenzyl derivatives **3** became the main products. Besides the monoalkylated product, even dialkylated compounds (albeit in negligible amounts) were detected by the LC/MS analysis.

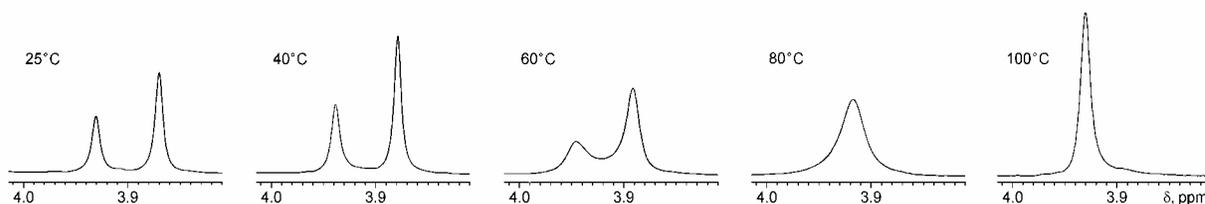
A detailed NMR investigation of the side product – the presumed trifluoroacetate **3** – was performed using DQF-COSY [4-6], long range INEPT [7] and COLOC [8-10] experiments. At room temperature, the molecule has two major conformations in equilibrium (conformation A: conformation B = 3: 2), a fact most obviously observable by the presence of doubled signals of H-4, H-17, NH<sub>3</sub><sup>+</sup> and NH protons. Signal coalescence was achieved in variable temperature <sup>1</sup>H- and <sup>13</sup>C- NMR experiments (see Experimental).

The activation energy of the exchange process ( $\Delta G^\ddagger = 75$  kJ/mol) was measured from signal coalescence of proton H-17 in VT <sup>1</sup>H-NMR experiments, using the formula below, applicable to 1:1 exchange processes [11].

$$\Delta G^\ddagger = RT_c[22.96 + \ln(T_c/\Delta \nu)]$$

The broad <sup>1</sup>H peaks of the NH<sub>3</sub><sup>+</sup> and NH groups groups coalesced at 60°C; the signals of the benzylic H-4 and H-17 of the two conformers coalesced at 80°C (Figure 1).

**Figure 1.** Variable temperature <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) spectra for H-17a/b of compound **3**.



NMR investigations were made at 25-40-60-80-100-120°C. The best resolved <sup>1</sup>H- spectra were obtained at 100°C. Doubled carbon peaks were observed for the indole and 4-hydroxybenzyl rings even at this high temperature. At 120°C decomposition of the molecule precluded long time NMR investigations. The largest chemical shift changes during both VT <sup>1</sup>H- and <sup>13</sup>C- measurements were observed for the atoms of the phenol ring and of the indole ring system while the benzylic ring gave

single peaks even in room temperature investigations. Hence, the doubling of peaks at low temperature might be caused by the presence of two different low energy conformations of the 4-hydroxybenzyl group.

In long range heteronuclear shift correlation experiments (COLOC [8-10] at 25°C, at 80°C and at 120°C, long range INEPT [7] at 25°C) H-17 gave three strong and one weak aromatic cross peaks [111.0 ppm weak (C-15); 129.7 ppm (C-19 and C-23); 130.5 ppm (C-18); 135.4 ppm (C-16) and an aliphatic cross peak through one bond correlation at 31.5 ppm (C-17)]. These results confirm the suggested structure **3**.

## Conclusions

We have described a side reaction occurred when using carboxylated Wang polymer for the synthesis of indole derivatives. We could unambiguously assign <sup>1</sup>H- and <sup>13</sup>C- resonances from one of these side products' NMR spectra, proving that a *p*-hydroxybenzylation reaction proceeds at position 2 of the indole ring. Elevated concentrations of 1,2-ethanedithiol in the cleavage mixtures efficiently reduced the side product formation.

## Experimental

### General

Reagents were obtained from Aldrich or Fluka with following exceptions: Wang resin was purchased from Bapeks, Latvia, and PyBroP was a product of Novabiochem. LC/MS was performed on a Perkin Elmer PE SCIEX API 150EX instrument equipped with a Turboionspray Ion Source and a Dr. Maisch Reprosil–Pur C18-AQ, 5 μ, 150 x 3 mm HPLC column, using a gradient formed from water and acetonitrile with 5 mM ammonium acetate additive. Semipreparative HPLC was carried out on a LKB system consisting of a 2150 HPLC Pump, 2152 LC Controller and 2151 Variable Wavelength Monitor (manual collection of eluate fractions corresponding to peaks). Freeze-drying was performed at 0.01 bar on a Lyovac GT2 Freeze-Dryer (Finn-Aqua) equipped with a Trivac D4B (Leybold Vacuum) vacuum pump and a liquid nitrogen trap. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded using a Jeol EX-400 or Jeol EX-270 instruments. High resolution mass spectra were obtained on a Q-Tof-2 (Micromass) spectrometer.

*N*-(3-Amino-propyl)-*N*-benzyl-4-(1*H*-indol-3-yl)-butyramide (**2**) trifluoroacetate and *N*-(3-Amino-propyl)-*N*-benzyl-4-[2-(4-hydroxy-benzyl)-1*H*-indol-3-yl]-butyramide (**3**) trifluoroacetate.

Wang resin (0.64 g, 0.7 mmole) was suspended in CH<sub>2</sub>Cl<sub>2</sub> (5 mL), *p*-nitrophenylchloroformate (0.42 g, 2.1 mmole) was added, and the mixture was cooled to 0°C. A solution of *N*-methylmorpholine (0.5 mL, 4.0 mmole) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was added in small portions with shaking. Then the reaction

mixture was allowed to warm to room temperature and gently agitated for 4 hours. The resin was filtered off, washed successively with DMF (6 x 4 mL), MeOH (3 x 4 mL) and CH<sub>2</sub>Cl<sub>2</sub> (2 x 4 mL) and dried in a dessicator. The *p*-nitrophenyl carbonate Wang resin obtained (0.84 g, 0.7 mmole) was placed in a reaction vessel (size 15 mL), a solution of 1,3-diaminopropane (0.29 mL, 3.5 mmole) in DMF (5 mL) was added and agitated for 24 hours at room temperature. Then the mixture was filtered, the resin washed with DMF, MeOH and CH<sub>2</sub>Cl<sub>2</sub> (each solvent 3 x 5 mL) and dried in vacuum. To the 1,3-diaminopropane resin obtained a solution of benzaldehyde (0.37 g, 3.5 mmole) in trimethyl orthoformate (5 mL) was added and agitated 20 hrs at room temperature. Then the mixture was filtered, the resin washed with CH<sub>2</sub>Cl<sub>2</sub> (3 x 5 mL) and dried under vacuum. Sodium cyanoborohydride (220 mg, 3.5 mmole) was added, carefully mixed with the resin, then 4% acetic acid in trimethyl orthoformate (6 mL) was added in one portion and immediately intensively shaken for 5 min. Then the mixture was filtered, the resin was washed with MeOH, water, MeOH again and CH<sub>2</sub>Cl<sub>2</sub> (each solvent 3 x 5 mL), and 10% solution of diisopropylethylamine in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) was added and agitated for 10 min at room temperature. The mixture was filtered again and the resin was washed with CH<sub>2</sub>Cl<sub>2</sub> (6 mL). 3-indolebutyric acid (213 mg, 1.05 mmole), PyBroP (489 mg, 1.05 mmole) and diisopropylethylamine (359 µL, 1.05 mmole) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) were added and agitated for 20 hrs at room temperature. The mixture was filtered, the resin washed with DMF, MeOH and CH<sub>2</sub>Cl<sub>2</sub> (each solvent 3 x 5 mL), and dried in vacuum. Then the resin was treated first with 1,2-ethanedithiol (1.2 mL), then water (150 µL), triisopropylsilane (150 µL) and trifluoroacetic acid (6 mL) were added. The reaction mixture was gently agitated at room temperature for 1 hour, filtered and the resin on the filter washed with trifluoroacetic acid (3 mL). The combined filtrate was evaporated *in vacuo* at room temperature and the residue treated with dry ether. A white crystalline precipitate formed which was filtered off and washed on the filter with dry ether. Then the substance obtained was dried *in vacuo* in presence of KOH and P<sub>2</sub>O<sub>5</sub>. The raw product was further dissolved in 30 % MeCN in water, centrifuged and the clear solution applied onto an HPLC semipreparative column (10 x 250 mm, Vydac 219TP510 Diphenyl), eluent - 32 % MeCN in water + 0.1% TFA, detection at 280 nm. Eluate fractions, containing pure putative **2** and **3** trifluoroacetates were separately pooled and lyophilized. White powders formed. Yield of **2** trifluoroacetate: 31 mg (9.6 %). Yield of **3** trifluoroacetate: 4.7 mg (1.2 %). Cleavage excluding 1,2-ethanedithiol: yield of **2** and **3** trifluoroacetates was 1.5 % and 10.5 % respectively. For analytical purposes **2** and **3** trifluoroacetates were converted to corresponding hydrochlorides. (**2** or **3** trifluoroacetate solution in methanol was slowly passed through a small column filled with Dowex 1 x 4 in Cl<sup>-</sup> form. The eluate was evaporated and the residue treated with anhydrous ether to yield **2** or **3** hydrochloride.)

#### Variable Temperature NMR Studies

<sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, 25 °C): δ 1.68-1.90 (m, I=4, 4H<sub>A</sub>+ 4H<sub>B</sub>, H-2<sub>A</sub>, H-2<sub>B</sub>, H-13<sub>A</sub>, H-13<sub>B</sub>), 2.33 (t, J=7.4 Hz, I=1, 1H<sub>A</sub>, H-12<sub>A</sub>), 2.45 (t, J=6.8 Hz, I=0.7, 1H<sub>B</sub>, H-12<sub>B</sub>), 2.67 (t, J=7.2 Hz, I=1, 1H<sub>A</sub>, H-1<sub>A</sub>), 2.75 (m, I=2.4, 1H<sub>A</sub>, 2H<sub>B</sub>, H-1<sub>B</sub>, H-14<sub>A</sub>, H-14<sub>B</sub>), 3.27 (t, J=8.0 Hz, I=0.7, 1H<sub>B</sub>, H-3<sub>B</sub>), 3.34 (t,

J=7.0 Hz, I=1, 1H<sub>A</sub>, H-3<sub>A</sub>), 3.86 (s, I=1, 1H<sub>A</sub>, H-17<sub>A</sub>), 3.92 (s, I=0.7, 1H<sub>B</sub>, H-17<sub>B</sub>), 4.48 (s, I=1, 1H<sub>A</sub>, H-4<sub>A</sub>), 4.52 (s, I=0.7, 1H<sub>B</sub>, H-4<sub>B</sub>), 6.67 (t, J=8.8 Hz, I=2, ArH, H-20/22), 6.92 (t, J=7.7 Hz, I=1, ArH, H-27), 6.98 (m, I=3, ArH, H-19/23, H-26), 7.07 (m, I=2, ArH, H-6/10), 7.23 (m, I=1, ArH, H-25), 7.3 (m, I=1, ArH, H-8), 7.35 (m, I=2, 2H, ArH, H-7/9), 7.44 (m, I=1, ArH, H-28), 7.79 (s, I=2, 3H<sub>A</sub>, NH<sub>3</sub><sup>+</sup><sub>A</sub>), 7.88 (s, I=1, 3H<sub>B</sub>, NH<sub>3</sub><sup>+</sup><sub>B</sub>), 9.32 (s, I=1, Ar-OH), 10.7 (s, I=0.7, NH<sub>A</sub>), 10.72 (s, I=0.3, NH<sub>B</sub>).

<sup>13</sup>C-NMR (100 MHz, DMSO-d<sub>6</sub>, 25 °C): δ 23.7 (C-1), 23.9 (C-2/13), 26.0, 26.8, 26.9, 27.1, 31.3 (C-17), 32.2 (12A), 32.6 (C-12), 37.0, 37.3 (C-14), 43.2 (C-3), 44.6 (C-3), 48.0 (C-4), 51.0 (C-4), 60.8, 72.8, 110.6 (C-15), 110.9, 111.2 (C-25), 115.7 (C-20/22), 118.4 (C-28), 118.6 (C-27), 120.7 (C-26), 126.9 (C-6/10), 127.5, 127.7(C-8), 127.8, 128.6 (C-24), 128.7, 128.9, 129.3 (C-7/9), 129.7 (C-19/23), 129.8 (C-19/23), 130.5 (C-18), 130.6, 135.4 (C-16), 136.0, 138.1 (C-5A), 138.8 (C-5B), 156.1, 158.6, 158.9 (C-21), 172.7 (C-11A), 173.8 (C-11B).

<sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>, 100 °C): δ 1.70-1.90 (m, I=4, 4H, H-2, H-13), 2.39 (t, J=6.6 Hz, I=2, 2H, H-12), 2.73 (t, J=7.6 Hz, I=2, 2H, H-1), 2.79 (t, J= 6.8 Hz I=2, 2H, H-14), 3.36 (t, J=7 Hz, I=2, 2H, H-3), 3.94 (s, I=2, 2H, H-17), 4.51 (s, I=2, 2H, H-4), 6.69 (AA' part of AA'XX', I=2, ArH, H-20/22), 6.93 (C part of ABCD, I=1, ArH, H-27), 7.01 (m, I=3, ArH, H-19/23, H-26), 7.18 (A part of ABC, I=2, ArH, H-6/10), 7.27 (m, I=2, ArH, H-25, H-8), 7.34 (B part of ABC, I=2, 2H, ArH, H-7/9), 7.44 (D part of ABCD, J=7.7 Hz, I=1, ArH, H-28), 7.75 (s, I=3, NH<sub>3</sub><sup>+</sup>), 9.00 (bs, I=1, Ar-OH), 10.35 (bs, I=1, NH).

<sup>13</sup>C-NMR (100MHz, DMSO-d<sub>6</sub>, 100 °C): δ 24.0 (C-1), 26.5 (C-2/13), 26.9 (C-13/2), 31.5 (C-17), 32.8 (C-12), 37.5 (C-14), 44.0 (C-3), 51.0 (C-4), 111.0 (C-15), 111.2 (C-25A), 111.3 (C-25B), 115.8 (C-20/22A), 115.9 (C-20/22B), 118.2 (C-28A), 118.3 (C-28B), 118.4 (C-27A), 118.5 (C-27B), 120.6 (C-26A), 120.7(C-26B), 126.9 (C-6/10), 127.5 (C-8), 127.7 (C-7/9), 128.9 (C-24A), 129.0 (C-24B/29), 129.6 (C-19/23A), 129.7 (C-19/23B), 130.6 (C-18), 135.4 (C-16), 136.3 (C-5), 156.3 (C-21), 173.9 (C-11).

### Spectral Data

**Compound 2 hydrochloride:** IR: 2834, 1614, 1451, 1229, 1008, 741, 698, 630, 614, 583 cm<sup>-1</sup>; <sup>1</sup>H-NMR (270 MHz, DMSO-d<sub>6</sub>): δ 1.63-1.96 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>NCO, CH<sub>2</sub>CH<sub>2</sub>CO), 2.37 (t, J=7.3 Hz, 1H, CH<sub>2</sub>CO), 2.46 (t, J=6.9 Hz, 1H, CH<sub>2</sub>CO), 2.64 (t, J=7.9 Hz, 1H, CH<sub>2</sub>NH<sub>3</sub><sup>+</sup>), 2.6-2.8 (m, 3H, CH<sub>2</sub>NH<sub>3</sub><sup>+</sup>, CH<sub>2</sub>-indole), 3.20-3.37 (m, 2H, CH<sub>2</sub>NCO), 4.50, 4.52 (2 s, 2H, CH<sub>2</sub>Ph), 6.9-7.55 (m, 10H, indole and benzene CH), 7.68 (br s, 3H, NH<sub>3</sub><sup>+</sup>), 10.74, 10.77 (2d, J=2.3Hz, 1H, NH); HRMS (ES) for C<sub>22</sub>H<sub>28</sub>N<sub>3</sub>O<sub>2</sub> (MH<sup>+</sup>): Calcd. 350.2232; Found 350.2233.

**Compound 3 hydrochloride:** IR 3029, 1676, 1613, 1512, 1453, 1358, 1200, 1132, 1010, 835, 798, 722, 698, 605, 579, 556 cm<sup>-1</sup>; HRMS (ES) for C<sub>29</sub>H<sub>34</sub>N<sub>3</sub>O<sub>2</sub> (MH<sup>+</sup>): Calcd. 456.2651; Found 456.2668.

## References

1. Riniker, B.; Kamber, B. In *Peptides 1988*; Jung, G.; Bayer, E., Eds.; Walter de Gruyter & Co: Berlin, New York, 1989; pp 115-117.
2. Giraud, M.; Cavelier, F.; Martinez., J. A side-reaction in the SPPS of Trp-containing peptides. *J. Pept. Sci.* **1999**, *5*, 457-461.
3. Prohazka, Z. In *Paper Chromatography. A Comprehensive Treatise*, Hais, I.M.; Macek, K., Eds.; Publishing House of the Czechoslovak Academy of Sciences: Prague, 1963; p.247.
4. Wokaun, A.; Ernst, R. R. Selective detection of multiple quantum transitions in NMR by two-dimensional spectroscopy. *Chem Phys. Lett.* **1977**, *52*, 407-412.
5. Piantini, U.; Sørensen, O. W.; Ernst, R. R. Multiple quantum filters for elucidating NMR coupling networks. *J. Am. Chem. Soc.* **1982**, *104*, 6800-6801.
6. Shaka, A.J.; Freeman, R. Simplification of NMR spectra by filtration through multiple-quantum coherence. *J. Magn. Reson.* **1983**, *51*, 169-173.
7. Bax, A. Structure determination and spectral assignment by pulsed polarization transfer via long-range proton-carbon-13 couplings. *J. Magn. Reson.* **1984**, *57*, 314-318.
8. Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H. R. Assignment of carbonyl carbons and sequence analysis in peptides by heteronuclear shift correlation via small coupling constants with broadband decoupling in t1 (COLOC). *J. Magn. Reson.* **1984**, *57*, 331-336.
9. Kessler, H.; Griesinger, C.; Lautz, J. Determination of connectivities via small proton-carbon couplings with a new two-dimensional NMR technique. *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 444-445.
10. Kessler, H.; Griesinger, C.; Wagner, K. Peptide conformations. 42. Conformation of side chains in peptides using heteronuclear coupling constants obtained by two-dimensional NMR spectroscopy. *J. Am. Chem. Soc.* **1987**, *109*, 6927-6933.
11. Günther, H. *NMR spectroscopy; Basic Principles, Concepts, and Applications in Chemistry*, 2<sup>nd</sup> Edition; John Wiley & Sons, Chichester, England, 1994.

*Samples Availability:* Samples not available.