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# Chemoenzymatic Synthesis of Epoxidized Methacrylamides Involving Glucoseoxidase/Glucose

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# CHEMOENZYMATIC SYNTHESIS OF EPOXIDIZED METHACRYLAMIDES INVOLVING GLUCOSEOXIDASE/GLUCOSE

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## INTRODUCTION

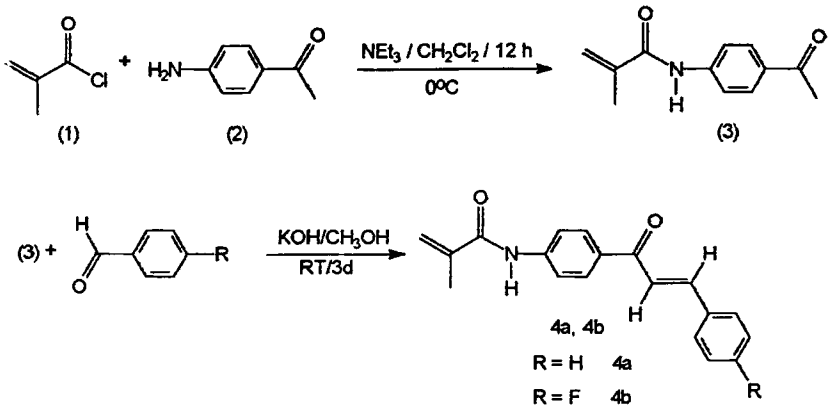
Recently, increasing interest has been spent on the use of suitable enzymes as catalysts for the synthesis of vinylmonomers, some oligomers and for the construction and modification of synthetic polymers[1]. In this field of research, mainly condensation reactions have been successfully performed in the presence of enzymes that are esterase's, lipase's or peptidases. The enzymatic generation of hydrogenperoxide in mixtures containing glucoseoxidase/glucose just has been applied for the initiation of free radical polymerization and for the degradation of some water-soluble polymers[2,3]. Usually, the enzyme glucoseoxidase is used in medical diagnostic devices for a quantitative determination of glucose content in human blood[4].

Up to now, it has not been described to use of this enzymatically produced hydrogenperoxide to epoxidize unsaturated monomers containing propenone functions in a preparative scale. Thus, in the present paper some results are presented dealing with the synthesis and chemoenzymatic epoxidation of chalcon modified methacrylmonomers.

## RESULTS AND DISCUSSION

*Synthesis of monomers*

Reaction of methacryloyl chloride (**1**) with 4-aminoacetophenone (**2**) in methylene chloride at 0°C gave the intermediate N-methacryloyl-4-aminoacetophenone (**3**) that was further condensed with benzaldehyde and 4-fluorobenzaldehyde yielding the polymerizable chalcon derivatives **4a** and **4b**. As a typical example, the $^1$H-NMR spectrum of **4a** is shown in Fig.1 that illustrates also an extended region from 6,5 to 8,0 ppm. The spectrum proves the *trans*-




Scheme 1

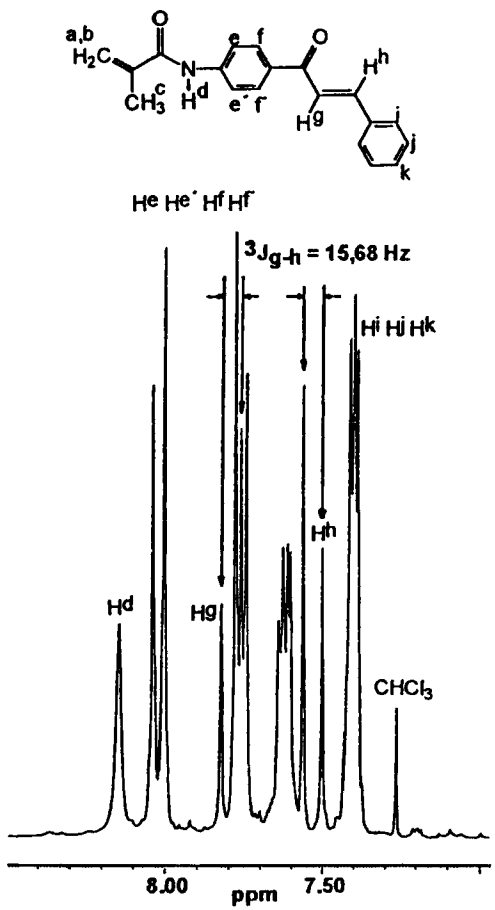



Fig.1: $^{1}$H-NMR-spectrum of **4a**, extended region from 7,0 to 8,4 ppm (250 MHz, $CDCl_3$)

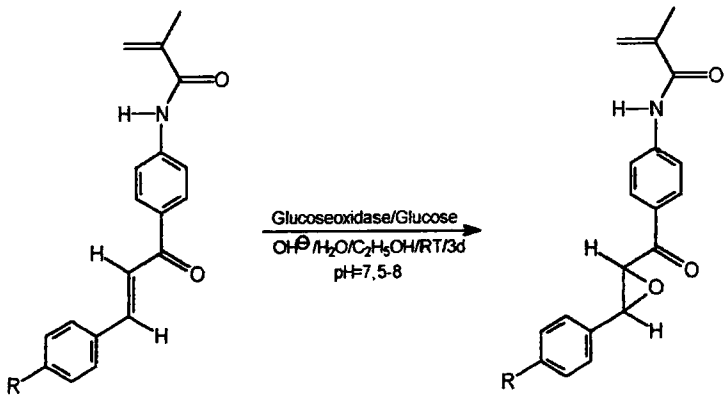
configuration of the chalcon function, because the $^3J$ coupling constant of the olefinic protons of the chalcon function appears to be above 15 Hz.

*Chemoenzymatical epoxidation of the chalcones **4a** and **4b***

In principle, the chemical epoxidation of chalcones with hydrogenperoxide or with *t*-butyl-hydroperoxide is well know and has been described in many papers[5]. As expected, the methacrylic monomers **4a** and **4b** can also be epoxidized with $H_2O_2$ yielding the corresponding oxirane derivatives **5a** and **5b.** Both substrates still contain the unmodified methacrylic function. From NMR study it is demonstrated that the epoxidized chalcon is still in a *trans*-configuration, because the coupling constant of the oxirane-protons is below 2 Hz[6].

As a new approach, the glucoseoxidase/glucose in a weakly alkaline alcohol/water-mixture was used to produce epoxidizing hydrogenperoxide in situ. It was clearly shown that this biological mixture is indeed able to produce the same epoxidized monomers **5a** and **5b** in a preparative scale. However, the speed of epoxidation is much slower than the above described chemical method. This indicates that the enzyme catalyzed hydrogenperoxide formation is relatively slow under the applied conditions.

The epoxidized chalcones **5a** and **5b** can be polymerized by free radical mechanism with




R = H 4a
R = F 4b

R = H 5a
R = F 5b

Scheme 2

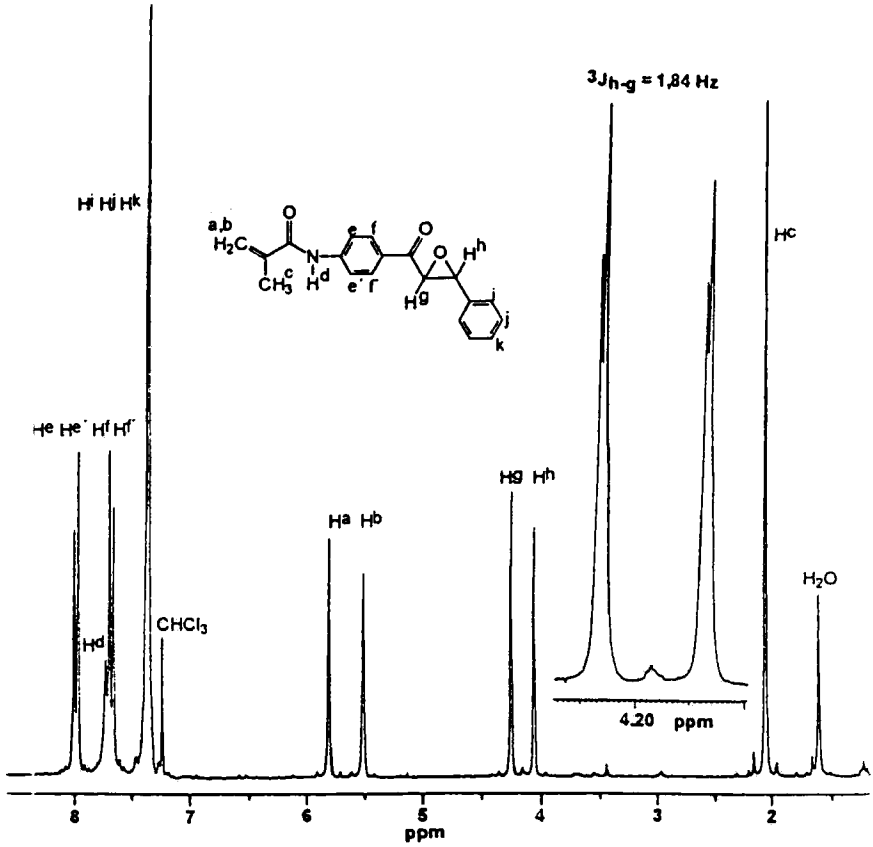



**Fig. 2:** $^1$H-NMR-spectrum of **5a** (250 MHz, $CDCl_3$)

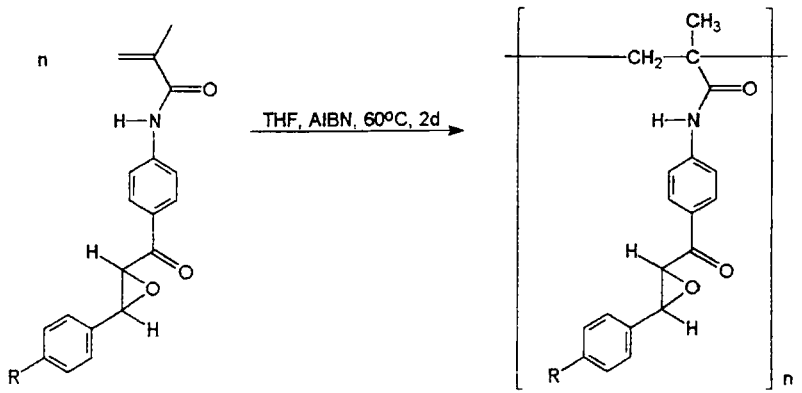
AIBN as initiator yielding the corresponding homopolymers **6a** and **6b**. The obtained polymers were characterized spectroscopically and by viscosity measurements.

The epoxidized chalcones **5a** and **5b** can be polymerized by free radical mechanism with AIBN as initiator yielding the corresponding homopolymers **6a** and **6b**. The obtained polymers were characterized spectroscopically and by viscosity measurements.

## EXPERIMENTAL PART

*Materials*

Methacryloyl chloride (**1**), 4-aminoacetophenone (**2**), hydrogenperoxide solution (30%), 2,2'-azoisobutyronitrile (AIBN), tetrahydrofuran (THF), chloroform-*d* ($CDCl_3$) and dimethyl




Scheme 3



sulfoxide-$d_6$ (DMSO-$d_6$)are commercially available (FLUKA).
The solvents were purified by standard methods[7]

*Measurements*

The NMR spectra were recorded with Bruker AC 250 and Bruker ARX 400, IR spectra with Perkin-Elmer 1420 and the mass spectra with Varian MAT 311 A (70 eV). The elemental analyses were performed with a Perkin-Elmer 204 B elemental analyser, the melting points with a Büchi Melting Point Determinator 510. The DSC measurements were carried out with a Perkin-Elmer DSC 7 differential scanning calorimeter, the TG measurements with a Mettler TA 300 in air and the viscometric measurements with an Ostwald viscometer in THF at 25 °C thermostated by Haake W 13.

*Synthesis of monomers*

*N-Methacryloyl-4-aminoacetophenone* (3)

To a mixture of 5,0 g (36,99 mmol) **2**, 6,0 mL of triethylamine and 30 mL of methylene chloride was added at 0 °C a solution of 3,7 mL (37 mmol) of **1** in 5 mL methylene chloride. The solution was stirred at room temperature for 3h, poured into 250 g of ice and then neutralized with 2N hydrochloride acid. The precipitated material was filtered off, washed three times with 30 mL of water and recrystallized from 40 mL of ethanol, yielding a colourless solid product. Yield: 6,80 g (90%), m.p.: 137 °C.

IR (KBr): ν = 3320 (NH), 2990 (CH), 1680 (–C=O amide I), 1630 (C=C, alkene) 1610,1595 (aromat.),1520 (NH ,amide II), 1410 (CH-def.), 840 $cm^{-1}$ (1,4-disubst. aromat.).

$^1$H-NMR ($CDCl_3$, 250 MHz): δ = 2,06 (s, 3H, $H_2C=C(C\underline{H}_3)$), 2,57 (s, 3H, $-C(O)C\underline{H}_3$), 5,52; 5,82 (ps, 2H, $\underline{H}_2C=$), 7,66-7,96 (m, 4H, $-C_6\underline{H}_4-$), 7,81 (s, 1H, $-C(O)N\underline{H}-C_6H_4-$).

$^{13}$C{$^1$H}-NMR ($CDCl_3$, 100,6 MHz): δ = 19,03 (C3), 26,75 (C10), 119,76 (C6), 120,91 (C1), 129,98 (C7), 133,21 (C8), 140,99 (C2), 142,93 (C5), 167,45 (C4), 197,55 (C9).

MS (70 eV): m/z(%) = 203(7) [$M^+$]

| | | | | |
|---|---|---|---|---|
| $C_{12}H_{13}NO_2$ (203,24) | Calc. | C 70,92 | H 6,45 | N 6,89 |
| | Found | C 70,75 | H 6,61 | N 6,80 |

*N-[4-(3-Phenyl-acryloyl)-phenyl]-methacrylamide* (**4a**)

A mixture of 2,0 g (9,84 mmol) of **3**, 0,99 mL (9,84 mmol) of benzaldehyde and 12 mL of an basic methanolic solution (0,5 g KOH/100 mL methanol) was vigorously stirred 3d at room temperature in the absence of light. After neutralization with 2N acetic acid, the precipitation was filtered off, washed three times with 50 mL of water and recrystallized from 15 mL of ethanol yielding a pale yellow, crystalline product. Yield: 2,50 g (87%), m.p.: 162-163 °C.

IR (KBr): ν = 3340 (NH), 3060,3020 (=CH, arom.), 2920 (CH), 1680 (–C=O ,arom. ketone),1645 (–C=O, amide I), 1630 (C=C, alkene), 1600,1570 (arom.), 1515 (NH, amide II), 1405 (CH-def.), 970 (=CH-def., (E)-ethene), 830 (1,4-disubst. arom.), 760,690 $cm^{-1}$ (monosubst. arom.).

$^1$H-NMR ($CDCl_3$, 250 MHz): δ = 2,06 (s, 3H, $H_2C=C(C\underline{H}_3)$), 5,49; 5,84 (ps, 2H, $\underline{H}_2C=$), 7,40-7,65 (m, 5H, $-C_6\underline{H}_5$), 7,52 (d, $^3$J=15,68 Hz, 1H, $-C(O)CH=C\underline{H}-$), 7,79 (d, $^3$J=15,68 Hz, 1H, $-C(O)C\underline{H}=CH-$), 7,82-8,04 (m, 4H, $-C_6\underline{H}_4-$), 8,15 ( s, 1H, $-C(O)N\underline{H}-C_6H_4-$).

$^{13}$C{$^1$H}-NMR ($CDCl_3$, 62,89 MHz): δ = 18,55 (C3), 119,23 (C6), 120,46 (C1), 121,51 (C10), 128,28 (C13), 128,78 (C15), 129,73 (C14), 130,37 (C7), 133,57 (C8), 134,66 (C12), 140,43 (C2), 142,08 (C11), 144,45 (C5), 166,75 (C4), 188,87 (C9).

MS (70 eV): m/z(%) = 291(10) [$M^+$],

| | | | | |
|---|---|---|---|---|
| $C_{19}H_{17}NO_2$ (291,35) | Calc. | C 78,33 | H 5,88 | N 4,81 |
| | Found | C 78,05 | H 5,89 | N 5,07 |

*N-[4-[3-(4-Fluoro-phenyl)-acryloyl]-phenyl]-methacrylamide* (**4b**)

The synthesis and isolation was performed analogously to **4a**.

A mixture of 2,0 g (9,84 mmol) of **3**, 1,05 mL (9,84 mmol) of 4-fluorobenzaldehyde and 12

mL of an basic methanol solution was stirred for 3d yielding colourless needles. Yield: 2,75 g (90%), m.p.: 175-176 °C.

IR (KBr): ν = 3280 (NH), 3060 (=CH, arom.), 2920 (CH), 1660 (–C=O, amide I), 1630 (C=C, alkene), 1610,1590,1490 (arom.), 1530,1520 (NH, amide II), 1410 (CH-def.), 1220 (C–F), 980 (=CH-def., (E)-ethene), 830,810 $cm^{-1}$ (2x1,4-disubst. arom.).

$^1$H-NMR ($CDCl_3$, 400 MHz): δ = 2,08 (s, 3H, $H_2C=C(C\underline{H}_3)$), 5,51; 5,84 (ps, 2H, $\underline{H}_2C=$), 6,60-7,94 (m, 4H, $-C_6\underline{H}_4F$), 7,48 (d, $^3$J=15,64 Hz, 1H, –C(O)CH=C<u>H</u>–), 7,78 (d, $^3$J=15,68 Hz, 1H, –C(O)C<u>H</u>=CH–), 7,73-8,04 (m, 4H, $-C_6\underline{H}_4-$), 7,90 ( s, 1H, –C(O)N<u>H</u>–$C_6H_4$–).

**$^{13}$C{$^1$H}-NMR ($CDCl_3$, 100,6 MHz): δ = 19,04 (C3), 116,46 (C14, d, $^2J_{C-F}$ = 21,6 Hz), 119,73 (C6), 120,85 (C1), 121,90 (C10), 130,27 (C12), 130,68 (C13, d, $^3J_{C-F}$ = 8,8 Hz), 131,58 (C7), 134,19 (C8), 141,10 (C2), 142,53 (C11), 143,59 (C5), 164,42 (C15, d, $^1J_{C-F}$ = 251,7 Hz), 167,11 (C4), 189,10 (C9).**

MS (70 eV): m/z(%) = 309(17) [$M^+$]

| | | | | |
|---|---|---|---|---|
| $C_{19}H_{16}NO_2F$ (309,34) | Calc. | C 73,77 | H 5,21 | N 4,53 |
| | Found | C 73,62 | H 5,22 | N 4,93 |

*Chemoenzymatical epoxidation*

*2,3-Epoxy-1-oxo-3-phenyl-1-(4-methacryloylamino-phenyl)-propane* **(5a)**

A mixture of 300 mg of **4a** in 6 mL ethanol/water (5:1; v/v), 300 mg of glucoseoxidase in 1 mL water and 450 mg of β-D-glucose was stirred at room temperature. 0,15 mL of an 1N NaOH solution were added subsequently. The activity of enzyme was proved by KJ starck paper. After addition of 0,10 mL of an 1N NaOH, the solution was stirred 3d at room temperature and poured in 10 mL of water. The precipitate was filtered off, washed four times with 10 mL of KJ solution (3%) and recrystallized twice from 4 mL of ethanol/water (4:1; v/v) obtaining a colourless solid.

Yield: 210 mg (66%), m.p.: 93-95 °C.

IR (KBr): ν = 3300,3260 (NH), 3060 (=CH, arom.), 2970 (CH), 1660 (–C=O, amide I), 1620 (C=C, alkene), 1590 (arom.), 1520 (NH, amide II), 1405 (CH-def.), 885 (C–O–C def.), 820 $cm^{-1}$ (1,4-disubst. arom.), 755,695 $cm^{-1}$ (monosubst. arom.).

$^1$H-NMR ($CDCl_3$, 250 MHz): δ = 2,06 (s, 3H, $H_2C=C(C\underline{H}_3)$), 4,07 d; 4,26 d ($^3$J=1,80 Hz, 2H, –C<u>H</u>–O–C<u>H</u>–), 5,52; 5,82 (ps, 2H, $\underline{H}_2C=$), 7,38 (m, 5H, $-C_6\underline{H}_5$), 7,70-8,02 (m, 4H, $-C_6\underline{H}_4-$), 7,75 (s, 1H, –C(O)N<u>H</u>–$C_6H_4$–).

$^{13}C\{^1H\}$-NMR ($CDCl_3$, 62,89 MHz): $\delta$ = 18,55 (C3), 59,23 (C11), 60,73 (C10), 119,17 (C6), 120,60 (C1), 125,68 (C12), 128,65 (C13), 128,93 (C15), 129,79 (C14), 131,07 (C7), 135,38 (C8), 140,46 (C2), 142,88 (C5), 166,50 (C4), 191,51 (C9).

MS (70 eV): m/z(%) = 307(20) [$M^+$]

| | | | | |
|---|---|---|---|---|
| $C_{19}H_{17}NO_3$ (307,35) | Calc. | C 74,25 | H 5,58 | N 4,56 |
| | Found | C 74,06 | H 5,59 | N 4,22 |

*2,3-Epoxy-1-oxo-3-(4-fluoro-phenyl)-1-(4-methacryloylamino-phenyl)-propane* (**5b**)

The synthesis and isolation was performed analogously to **5a**.

To a stirred mixture of 350 mg of **4b** in 6 mL ethanol/water (5:1; v/v), 350 mg of glucoseoxidase in 1 mL water and 450 mg of $\beta$-D-glucose 0,15 mL of an 1N NaOH solution was added subsequently.

Yield: 250 mg (68%), m.p.: 126-128 °C.

IR (KBr): $\nu$ = 3300,3240 (NH), 3060 (=CH, arom.), 2970, (CH), 1660 (–C=O, amide I), 1620 (C=C, alkene), 1600,1580 (arom.), 1510 (NH, amide II), 1415, (CH-def.), 890 (C–O–C def.), 845,815 $cm^{-1}$ (2x 1,4-disubst. arom.),

$^1$H-NMR ($CDCl_3$, 400 MHz): $\delta$ = 2,07 (s, 3H, $H_2C=C(C\underline{H}_3)$), 4,07 d; 4,23 d ($^3J$=1,76 Hz, 2H, –C<u>H</u>–O–C<u>H</u>–), 5,53; 5,84 (ps, 2H, <u>H</u>$_2$C=), 7,07-7,36 (m, 4H, $-C_6\underline{H}_4F$), 7,72-8,01 (m, 4H, $-C_6\underline{H}_4-$), 7,93 (s, 1H, –C(O)N<u>H</u>–$C_6H_4$–).

$^{13}C\{^1H\}$-NMR ($CDCl_3$, 100,6 MHz): $\delta$ = 19,00 (C3), 59,07 (C11), 61,19 (C10), 116,20 (C14, d, $^2J_{C\text{-}F}$ = 22,0 Hz), 119,80 (C6), 121,05 (C1), 127,92 (C12), 128,01 (C8), 130,23 (C7), 131,68 (C13, d, $^3J_{C\text{-}F}$ = 8,5 Hz), 140,97 (C2), 143,37 (C5), 163,42 (C15, d, $^1J_{C\text{-}F}$ = 248,1 Hz), 167,11 (C4), 191,85 (C9).

MS (70 eV): m/z(%) = 325(6) [$M^+$]

| | | | | |
|---|---|---|---|---|
| $C_{19}H_{16}NO_3F$ (325,36) | Calc. | C 70,14 | H 4,96 | N 4,31 |
| | Found | C 69,96 | H 4,97 | N 4,19 |

*Synthesis of the polymers*

*Poly-[2,3-Epoxy-1-oxo-3-phenyl-1-(4-methacryloylamino-phenyl)-propane]* (**6a**)

A solution of 0,90 g (2,88 mmol) of **5a** and 24,0 mg (5 mol%) of AIBN in 6 mL of THF was stirred 2d at 60 °C under nitrogen. The viscous suspension was diluted with 5 mL of THF and dropped into 150 mL of diethyl ether. The resulting polymer was filtered off, washed with 25 mL of diethyl ether and dried i. vac. at 50 °C.

Yield: 0,85 g (94%), dec. p.: 255 °C (TG)

IR (KBr): ν = 3380 (NH), 3060 (=CH, arom.), 2990 (CH), 1680 (–C=O, amide I), 1590 (arom.), 1520 (NH, amide II), 1405 (CH-def.), 890 (–C–O–C– def.), 820 $cm^{-1}$ (1,4-disubst. arom.), 755, 695 $cm^{-1}$ (monosubst. arom.).

$^1$H-NMR (DMSO-$d_6$, 400 MHz): δ = 0,8-1,5 (br,C<u>H</u>$_3$), 1,8-2,4 (br,C<u>H</u>$_2$), 4,1; 4,6 (br, –C<u>H</u>–O–C<u>H</u>–), 7,3 (br,$C_6$<u>H</u>$_5$–), 7,5-7,9 (–$C_6$<u>H</u>$_4$–), 9,3 (br, N<u>H</u>).

$\eta_i$ /c = 23,47 [$10^{-3}$ L/g]; c = 2,5 g/L; THF, 25 °C

| | | | | |
|---|---|---|---|---|
| $(C_{19}H_{17}NO_3)_n$ (307,35)$_n$ | Calc. | C 74,25 | H 5,58 | N 4,56 |
| | Found | C 72,56 | H 5,02 | N 4,12 |

*Poly-[2,3-Epoxy-1-oxo-3-(4-fluoro-phenyl)-1-(4-methacryloyl-amino-phenyl)-propane]* (**6b**)
A solution of 1,00 g (3,07 mmol) of **5b** and 25,2 mg (5 mol%) of AIBN in 8 mL of THF was stirred 2d at 60 °C under nitrogen. The viscous suspension was diluted with 5 mL of THF and dropped into 200 mL of diethyl ether. The resulting polymer was filtered off, washed with 25 mL of diethyl ether and dried i. vac. at 50 °C. Yield: 0,90 g (90%), dec. p.: 264 °C (TG)

IR (KBr): ν = 3300 (NH), 3060 (=CH, arom.), 2980, (CH), 1650 (–C=O, amide I), 1600-1580 (arom.), 1510 (NH , amide II), 1420, (CH-def.), 885 (–C–O–C– def.), 830 $cm^{-1}$ (2× 1,4-disubst. arom.),

$^1$H-NMR (DMSO-$d_6$, 400 MHz): δ = 0,8-1,4 (br,C<u>H</u>$_3$), 1,6-2,4 (br,C<u>H</u>$_2$), 4,0; 4,6 (br, –C<u>H</u>–O–C<u>H</u>–), 7,1-7,3 (br,$C_6$<u>H</u>$_4$F–), 7,5-8,1 (–$C_6$<u>H</u>$_4$–), 9,4 (br, N<u>H</u>).

$\eta_i$ /c = 23,51 [$10^{-3}$ L/g]; c = 2,5 g/L; THF, 25 °C

| | | | | |
|---|---|---|---|---|
| $(C_{19}H_{16}NO_3F)_n$ (325,36)$_n$ | Calc. | C 70,14 | H 4,96 | N 4,31 |
| | Found | C 67,89 | H 5,26 | N 4,24 |

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