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Macromolecular prodrugs. IX. Synthesis of polymer-fenoprofen conjugates

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Abstract

Synthesis of several polymer-fenoprofen conjugates is described. Fenoprofen was first chemically modified into benzotriazolide **2** and amino acid amide derivatives: glycine fenoprofenamide (**3a**) and β-alanine fenoprofenamide (**3b**) and their benzotriazolides **6a** and **6b**. Compounds **2** and **6** readily reacted with polyhydroxy aspartamide-type polymers, i.e. poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)] (PHEA) and poly[α,β-(*N*-3-hydroxypropyl-DL-aspartamide)] (PHPA) forming conjugates **5, 8a**,**b** and **9a**,**b**, respectively. Conjugate **11** was obtained by partial aminolysis of poly-DL-(2,5-dioxo-1,3-pyrrolidinediyl) (PSI) with 2-aminoethyl fenoprofenamide (**3c**), followed by total aminolysis with 2-hydroxyethylamine. The synthesised polymer-drug conjugates differed in type of covalent bounding, type and/or length of spacer and drug-loading.

Keywords: Fenoprofen; Polymer-drug conjugate; Macromolecular prodrug; Spacer; Poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]; Poly[α,β-(*N*-3-hydroxypropyl-DL-aspartamide)]; Poly[α,β-(*N*-2-aminoethyl-DL-aspartamide]-poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-copolymer

Abbreviations: L-Asp, L-aspartic acid; Bt, *N*-1-benzotriazolyl; BtH, benzotriazole; DMF, *N*,*N*-dimethylformamide; Fen, fenoprofen residue without carboxylic group; NSAID, non-steroidal anti-inflammatory drug; PAHA, poly[α,β-(*N*-2-aminoethyl-DL-aspartamide)]-poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-copolymer; PAHA-Fen, poly[α,β-(*N*-2-aminoethyl-DL-aspartamide)]-poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide]-copolymer-fenoprofen conjugate; PHEA, poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]; PHEA-β-Ala-Fen, poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-fenoprofen conjugate with β-alanine spacer; PHEA-Gly-Fen, poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-fenoprofen conjugate with glycine spacer; PHPA, poly[α,β-(*N*-3-hydroxypropyl-DL-aspartamide)]; PHPA-β-Ala-Fen, poly[α,β-(*N*-3-hydroxypropyl-DL-aspartamide)]-fenoprofen conjugate with β-alanine spacer; PHPA-Gly-Fen, poly[α,β-(*N*-3-hydroxypropyl-DL-aspartamide)]-fenoprofen conjugate with glycine spacer; PSI, poly-DL-(2,5-dioxo-1,3-pyrrolidinediyl); TEA, triethylamine.

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1. Introduction

Fenoprofen is a well-known analgesic and non-steroidal anti-inflammatory drug (NSAID) which is used in the management of mild to moderate pain, fever and inflammation associated with musculoskeletal and joint disorders such as osteoarthritis, rheumatoid arthritis and ankylosing spondylitis (Brooks et al., 1996). Prolonged therapy with fenoprofen and other NSAIDs may cause gastrointestinal ulceration and haemorrhage. In addition, fenoprofen has rather short plasma half-life (2–3 h), so repeated doses must be given to maintain a therapeutic effect. To overcome these problems, numerous prodrugs and structural analogues of NSAIDs and fenoprofen itself have been synthesised and tested for their analgesic/anti-inflammatory activity and gastrointestinal toxicity (see for example: Whitehouse and Rainsford, 1980; Myhren et al., 1998; Hellberg et al., 1998; Uegama et al., 1999). Prodrugs are one approach that can lead both to prolonged pharmacological activity and reduced adverse effects, and additionally, to increased water solubility or lipophilicity, improved site-specificity and patient acceptance (Wermuth et al., 1996). Polymer-drug conjugates, in which drugs are linked to polymeric matrices by covalent, cleavable bonds, are a promising type of prodrugs. In previous papers, binding of fenoprofen and analogous NSAIDs to biodegradable polysaccharides and poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)] (PHEA) by ester bonds has been published (Larsen and Johansen, 1989; Larsen et al., 1989; Giammona et al., 1991; Zorc et al., 1993; Zorc and Butula, 1994). Hydrolysis studies showed that drug could be released from the macromolecular prodrugs after chemical hydrolysis in wide pH range. The stability of PHEA-ketoprofen conjugate towards different enzymatic systems (Jakšić et al., 1996), probably caused by sterical hindrance, encouraged the design of conjugates with prolonged distance between main chain and drug component which would theoretically make enzyme–substrate interaction more favourable. In this paper, the attachment of fenoprofen to PHEA and PHPA by amide and ester bonds, through different non-toxic spacers is reported. Such prepared polymer-fenoprofen conjugates could be potentially useful fenoprofen prodrugs.

2. Materials and methods

2.1. Materials

IR spectra were recorded on a FT-IR Paragon 500 spectrometer (Perkin–Elmer, UK) and UV spectra on a Hewlett Packard 8452A Diode Array spectrophotometer (Hewlett Packard, Germany). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer (Varian, USA), operating at 75.5 MHz for the ^{13}C nucleus. Samples were measured in DMSO-d_6 solutions at 20 °C in 5-mm NMR tubes. Chemical shifts (ppm) are referred to TMS. DSC thermograms were taken with a Shimadzu DSC-50 instrument (Shimadzu, Japan). Dialysis was performed with Visking Dialysis Tubing (18/22 inch (Serva, Germany). For thin layer chromatography, silica gel sheets Kieselgel 60 F_{254} (Merck, Germany) were used. Solvent systems were dichloromethane/methanol (9.5:0.5), cyclohexane/ethyl acetate (1:1) and methanol. For spot detection iodine vapour was used. Column chromatography was performed on silica gel 0.063–0.200 mm (Kemika, Croatia), with dichloromethane/methanol (9.5:0.5) mixture and pure methanol as eluent. Fenoprofen was purchased from Eli Lilly Company (USA). The amines were distilled and dried prior to use. All solvents were of analytical grade purity and dry.

2.2. Chemistry

2.2.1. *N*-1-benzotriazolecarboxylic acid chloride (**1**)

The compound **1** was prepared according to the procedure published previously (Butula et al., 1977).

2.2.2. Fenoprofen benzotriazolide (**2**)

The compound **2** was prepared from fenoprofen and *N*-1-benzotriazolecarboxylic acid chloride (**1**), following the published procedure (Zorc and

Butula, 1994). ^{13}C NMR (DMSO-d_6), δ (ppm): 172.80 (C-1), 157.02 (C-6), 156.25 (C-1′), 145.50 (C-4), 141.89 (C-8″), 130.99 (C-7″), 130.78 (C-9″), 130.52 (C-8), 130.10 (C-3′,5′), 126.63 (C-6″), 123.72 (C-4′), 122.64 (C-9), 120.14 (C-5″), 118.81 (C-2′,6′), 118.09 (C-5), 117.20 (C-7), 114.07 (C-4″), 44.59 (C-2), 18.61 (C-3) (^{13}C NMR data for this compound have not been reported).

2.2.3. Glycine fenoprofenamide (**3a**) and β-alanine fenoprofenamide (**3b**)

Amino acids derivatives of fenoprofen were synthesised from benzotriazolide **2** and corresponding amino acid (Zovko et al., 2001).

2.2.4. 2-Aminoethyl fenoprofenamide (**3c**)

Solution of 1.030 g (0.003 mol) fenoprofen benzotriazolide (**2**) in 30 ml toluene was added dropwise within 30 min to a solution of 6 g (0.1 mol) ethylenediamine in 5 ml toluene. The reaction mixture was stirred for 1.5 h at room temperature and extracted two times with saturated NaCl solution and two times with water. The organic layer was dried over sodium sulphate, filtered and evaporated under reduced pressure. Yield: 0.830 g (98%). The analytically pure sample was obtained after column chromatography (mobile phase: dichloromethane/methanol (9:1) for elution of impurities and methanol for elution of the sticky product **3c.** IR (film): ν_{max} 3292, 1649, 1582, 1549, 1486, 1445, 1246, 1162, 931, 759, 694 cm^{-1}. ^{1}H NMR (DMSO-d_6), δ (ppm): 8.03 (t, 1H, $J = 5.3$ Hz, CONH), 7.38 (t, 2H, $J = 7.8$ Hz, H-2′,6′), 7.29 (t, 1H, $J = 7.8$ Hz, H-4′), 7.13 (t, 1H, $J = 7.4$ Hz, H-8), 7.09 (d, 1H, $J = 8.1$ Hz, H-7), 7.00 (*s*, 1H, H-5), 6.99 (d, 2H, $J = 7.7$ Hz, H-3′,5′), 6.83 (dd, 1H, $^3J = 8.0$ Hz, $^4J = 2.1$ Hz, H-9), 3.59 (q, 1H, $J = 7.0$ Hz, H-2), 3.06-2.98 (m, 2H, H-2″), 2.62 (bs, 2H, NH_2), 2.52 (t, 2H, $J = 6.6$ Hz, H-1″), 1.29 (d, 3H, $J = 6.9$ Hz, H-3). ^{13}C NMR (DMSO-d_6), δ (ppm): 173.11 (C-1), 156.66 (C-6), 156.56 (C-1′), 144.78 (C-4), 130.13 (C-3′,5′), 129.83 (C-8), 123.49 (C-4′), 122.44 (C-9), 118.56 (C-2′,6′), 117.61 (C-5), 116.63 (C-7), 45.02 (C-2), 42.18 (C-1″), 41.22 (C-2″), 18.61 (C-3). Elemental analysis for $C_{17}H_{20}N_2O_2$ (284.35) (%): calcd. C 71.81, H 7.09, N 9.85; found: C 71.46, H 7.42, N 9.45.

2.2.5. Poly[α,β-(N-3-hydroxypropyl-DL-aspartamide)] (PHPA, **4**)

To a solution of 3.100 g poly-DL-(2,5-dioxo-1,3-pyrrolidinediyl) (PSI, 10) in 16 ml *N*,*N*-dimethylformamide (DMF), 5.994 (0.0798 mol) of 3-hydroxypropylamine was slowly added. The reaction mixture was stirred at room temperature for 2.5 h and then acidified with diluted sulphuric acid to pH 4. The solution was diluted with water, dialysed against several changes of deionised water and lyophilised. Yield: 3.478 g (63%). IR (KBr): ν_{max} 3396, 3086, 2936, 2882, 1654, 1438, 1378, 1292, 1197, 1061, 1000, 658 cm^{-1} (Lovrek et al., 2000).

2.2.6. Poly[α,β-(N-3-hydroxypropyl-DL-aspartamide)]-fenoprofen conjugate (PHPA-Fen, **5**)

A solution of 0.585 g (0.0034 mol calculated as a monomer unit) PHPA (4), 0.584 g (0.0017 mol) **2** and 0.343 g (0.0034 mol) triethylamine (TEA) in 12 ml DMF was stirred at room temperature for 3 days. Solvent was evaporated under reduced pressure. The residue was triturated several times with acetone and ether in order to remove benzotriazole, unbound **2** and remaining DMF. The insoluble product **5** was filtered off. Yield: 0.417 g (42%). IR (KBr): ν_{max} 3292, 2937, 1737, 1656, 1538, 1483, 1240, 1171, 1069, 922, 756, 689 cm^{-1}. UV: λ_{max} 279 nm ($A = 0.423$; $\gamma = 130.4$ μg ml^{-1}, H_2O).

2.2.7. Benzotriazolide of glycine fenoprofenamide (**6a**)

To a solution of 0.308 g (0.0017 mol) chloride **1** in 20 ml toluene, a solution of 0.509 g (0.0017 mol) **3a** and 0.343 g (0.0034 mol) TEA in 20 ml toluene and 20 ml dioxane was added. The reaction mixture was stirred at room temperature for 2.5 h. TEA × HCl was filtered off and the mother liquor evaporated under reduced pressure. Crude **6a** was used in synthesis of **8a** and **9a** without further purification. A sample for spectroscopic analysis was obtained by extraction of toluene solution of **6a** once with aqueous TEA solution and three times with water. The organic layer was dried over sodium sulphate and evaporated. ^{1}H NMR (DMSO-d_6), δ (ppm): 8.82 (t, 1H, $J = 5.5$ Hz, NH), 8.26 (d, 1H, $J = 8.0$, Hz, H-4″), 8.18 (d,

1H, $J = 8.0$ Hz, H-7″), 7.78 (t, 1H, $J = 7.6$ Hz, H-5″), 7.61 (t, 1H, $J = 7.6$ Hz, H-6″), 7.39 (d, 2H, $J = 7.6$ Hz, H-2′,6′), 7.35 (dd, 1H, $J = 6.6$ Hz, H-4′), 7.15 (t, 1H, $J = 8.0$ Hz, H-9), 7.10 (s, 1H, H-5), 7.03 (d, 2H, $J = 8.6$ Hz, H-3′,5′, 6.99 (d, 1H, $J = 8.6$ Hz, H-7), 6.87 (dd, 1H, $J = 8.1$ Hz, H-8), 4.94 (d, 2H, $J = 5.5$ Hz, H-1″), 3.86 (q, 1H, $J =$ 7.0 Hz, H-2), 1.38 (d, 3H, $J = 7.0$ Hz, H-3). ^{13}C NMR (DMSO-d_6) δ (ppm): 174.15 (C-1), 168.72 (C-3″), 156.72 (C-6), 156.56 (C-1′), 145.34 (C-8″), 144.13 (C-4), 138.83 (C-9″), 128.97 (C-7″), 130.09 (C-3′,5′), 129.89 (C-8), 126.66 (C-6″), 123.44 (C-4′), 122.68 (C-9), 120.21 (C-5″), 118.63 (C-2′,6′), 117.96 (C-7), 116.87 (C-5), 114.99 (C-4″), 44.60 (C-2), 42.74 (C-1″), 18.73 (C-3).

2.2.8. Benzotriazolide of β-alanine fenoprofenamide (**6b**)

To a solution of 0.091 g (0.0005 mol) chloride **1** in 7 ml toluene, a solution of 0.157 g (0.0005 mol) **3b** and 0.051 g (0.0005 mol) TEA in 8 ml toluene was added dropwise. The reaction mixture was stirred at room temperature for 2 h and then extracted once with aqueous TEA solution and three times with water. The organic layer was dried over sodium sulphate, filtered and evaporated under reduced pressure. Yield: 0.197 g (95%). The analytically pure sample was obtained by triturating the oily product with petroleum ether. IR (film): ν_{max} 3287, 2973, 1750, 1641, 1591, 1549, 1482, 1448, 1382, 1267, 1242, 1210, 1168, 961, 754, 692 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.22 (t, 1H, $J = 5.3$ Hz, NH), 8.17 (d, 2H, $J = 8.7$ Hz, H-4″,7″), 7.33 (t, 1H, $J = 7.6$ Hz, H-5″), 7.57 (t, 1H, $J = 7.6$ Hz, H-6″), 7.34 (t, 2H, $J = 8.0$ Hz, H-2′,6′), 7.19, t, 1H, $J = 7.8$ Hz, H-8), 7.08 (t, 1H, $J = 7.3$ Hz, H-4′), 7.00 (d, 1H, $J = 7.7$ Hz, H-7), 6.95 (s, 1H, H-5), 6.93 (s, 2H, H-3′,5′), 6.74 (d, 1H, $J = 8.3$ Hz, H-9), 3.56 (m, 4H, H-2″,3″), 3.34 (s, 1H, H-2), 1.22 (d, 3H, $J = 7.0$ Hz, H-3). ^{13}C NMR (DMSO-d_6) δ (ppm): 173.32 (C-1), 170.84 (C-3″), 156.63 (C-6), 156.51 (C-1′), 145.55 (C-8″), 144.40 (C-4), 130.77 (C-7″,8), 130.63 (C-9″), 130.07 (C-3′,5′), 126.45 (C-6″), 123.44 (C-4′), 122.34 (C-9), 120.08 (C-5″), 118.62 (C-2′,6′), 117.63 (C-7), 116.58 (C-5), 114.10 (C-4″), 44.94 (C-2), 34.52 (C-2″), 35.34 (C-1″), 18.48 (C-3). Elemental analysis for $C_{24}H_{22}N_4O_3$ (414.46) (%): calcd. C 69.55, H 5.35, N 13.52; found: C 69.95, H 5.81, N 13.00.

2.2.9. Poly[α,β-(N-2-hydroxyethyl-DL-aspartamide)] (PHEA, **7**)

PHEA was prepared by aminolysis of PSI with 2-hydroxyethylamine (Neri et al., 1973, Zorc et al., 1992). To a solution of 6.200 g (0.0639) PSI (10) in 32 ml DMF, 9.773 (0.1600 mol) of 2-hydroxyethylamine was slowly added. The reaction mixture was stirred at room temperature for 2.5 h and then acidified with diluted sulphuric and glacial acetic acid to pH 4. The solution was diluted with water, dialysed against several changes of deionised water and lyophilised. Yield: 5.224 g (53 %). IR (KBr): ν_{max} 3428, 2926, 2856, 1646, 1538, 1434, 1379, 1238, 1061, 921, 853, 536 cm^{-1}.

2.2.10. Poly[α,β-(N-2-hydroxyethyl-DL-aspartamide)]-fenoprofen conjugate with glycine spacer (PHEA-Gly-Fen, **8a**)

A solution of 0.221 g (0.0014 mol calculated as a monomer unit) PHEA (7), 0.280 g (0.0007 mol) **6a** and 0.141 g (0.0014 mol) TEA in 15 ml DMF was stirred at room temperature for 3 days. Solvent was evaporated under reduced pressure. The residue was triturated several times with acetone and ether in order to remove benzotriazole, unbound **6a** and remaining DMF. The insoluble product **8a** was filtered off. Yield: 0.268 g (64%). IR (KBr): ν_{max} 3301, 3076, 2936, 1738, 1653, 1538, 1488, 1441, 1373, 1242, 1205, 1062, 928, 798, 694 cm^{-1}. UV: λ_{max} 272 nm ($A = 0.514$; $\gamma = 360$ μg ml^{-1}, ethanol/H_2O 2.5:1).

2.2.11. Poly[α,β-(N-2-hydroxyethyl-DL-aspartamide)]-fenoprofen conjugate with β-alanine spacer (PHEA-β-Ala-Fen, **8b**)

A solution of 0.380 g (0.0024 mol calculated as a monomer unit) PHEA (7), 0.497 g (0.0012 mol) **6b** and 0.242 g (0.0024 mol) TEA in 15 ml DMF was stirred at room temperature for 3 days. Solvent was evaporated under reduced pressure. The residue was triturated several times with acetone and ether in order to remove benzotriazole, unbound **6b** and remaining DMF. The insoluble product **8b** was filtered off. Yield: 0.334 g (46%).

IR (KBr): ν_{max} 3307, 3076, 2939, 1738, 1658, 1531, 1487, 1441, 1243, 1064, 930, 760, 692 cm^{-1}. UV: λ_{max} 272 nm ($A = 0.360$; $\gamma = 109.6$ μg ml^{-1}, ethanol/H_2O 2.5:1).

*2.2.12. Poly[α,β-(N-3-hydroxypropyl-DL-aspartamide)]-fenoprofen conjugate with glycine spacer (PHPA-Gly-Fen, **9a**)*

A solution of 0.241 g (0.0014 mol calculated as a monomer unit) PHPA (**4**), 0.280 g (0.0007 mol) **6a** and 0.141 g (0.0014 mol) TEA in 15 ml DMF was stirred at room temperature for 3 days. Solvent was evaporated under reduced pressure. The residue was triturated several times with acetone and ether in order to remove benzotriazole, unbound **6a** and remaining DMF. The insoluble product **9a** was filtered off. Yield: 0.263 g (60%). IR (KBr): ν_{max} 3298, 3074, 2942, 2880, 1734, 1653, 1540, 1490, 1438, 1374, 1242, 1200, 1069, 929, 758, 694 cm^{-1}. UV: λ_{max} 272 nm ($A = 0.591$; $\gamma = 392$ μg ml^{-1}, ethanol/H_2O 2.5:1).

*2.2.13. Poly[α,β-(N-3-hydroxypropyl-DL-aspartamide)]-fenoprofen conjugate with β-alanine spacer (PHPA-β-Ala-Fen, **9b**)*

A solution of 0.310 g (0.0018 mol calculated as a monomer unit) PHPA (4), 0.290 g (0.0007 mol) **6b** and 0.182 g (0.0018 mol) TEA in 12 ml DMF was stirred at room temperature for 3 days. Solvent was evaporated under reduced pressure. The residue was triturated several times with acetone and ether in order to remove benzotriazole, unbound **6b** and remaining DMF. The insoluble product **9b** was filtered off. Yield: 0.383 g (75%). IR (KBr): ν_{max} 3308, 3080, 2934, 1732, 1653, 1541, 1488, 1441, 1244, 1071, 930, 759, 693 cm^{-1}. UV: λ_{max} 272 nm ($A = 0.516$; $\gamma = 250$ μg ml^{-1}, ethanol/H_2O 2.5:1).

*2.2.14. Poly-DL-(2,5-dioxo-1,3-pyrrolidinediyl) (PSI, **10**)*

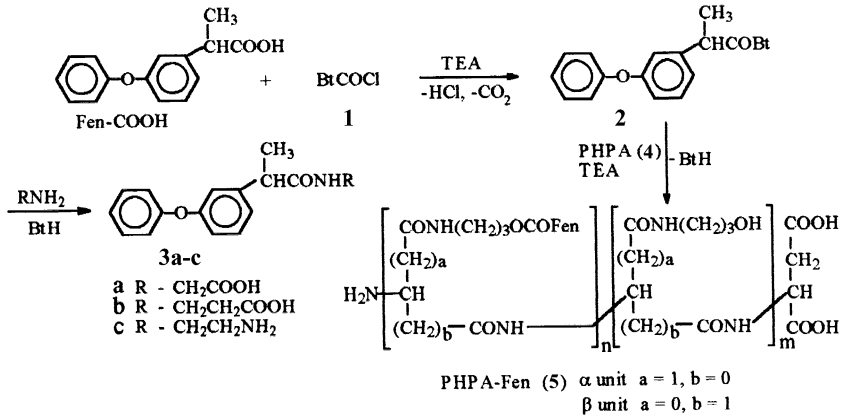
PSI was prepared by treating 14.800 g (0.1112 mol) L-aspartic acid (L-Asp) with 4 ml phosphoric acid, under reduced pressure in a rotary evaporator at 160 °C, for 2.5 h (Neri et al., 1973; Jakopović et al., 1996).

*2.2.15. Poly[α,β-(N-2-hydroxyethyl-DL-aspartamide)]-fenoprofen conjugate with ethylenediamine spacer (PAHA-Fen, **11**)*

A solution of 0.175 g (0.0018 mol calculated as a monomer unit) PSI (10) and 0.171 g (0.0006 mol) 2-aminoethyl fenoprofenamide (**3c**) in 15 ml DMF was stirred at room temperature 3 days. To this solution, 0.733 g (0.0120 mol) 2-hydroxyethylamine in 3 ml DMF was added dropwise. The reaction mixture was stirred at room temperature for 2.5 h and evaporated under reduced pressure. The residue was triturated several times with acetone and ether in order to remove unbound **3c**, excess of amine and remaining DMF. The insoluble product **11** was filtered off. Yield: 0.213 g (51%). IR (KBr): ν_{max} 3300, 3078, 2935, 2881, 1654, 1542, 1489, 1439, 1376, 1245, 1066, 932, 759, 694 cm^{-1}. UV: λ_{max} 272 nm ($A = 0.505$; $\gamma = 360$ μg ml^{-1}, H_2O).

3. Results and discussion

In the first step, fenoprofen reacted with *N*-1-benzotriazolecarboxylic acid chloride (**1**), an azole derivative very useful in preparation of different classes of organic compounds, e.g. esters, amides, ureas, hydantoins, carbamates, carbazides, oligopeptides, *N*-hydroxyisocyanate derivatives (see for example: Butula et al., 1983; Butula and Jadrijević-Mladar Takač, 2000). Thus prepared fenoprofen benzotriazolide (**2**) readily reacted with amino acids (glycine and β-alanine) or ethylenediamine, forming the amide derivatives with additional free functional group: carboxylic (**3a** and **3b**) or amino group (**3c**) (Scheme 1). Synthesis of glycine fenoprofenamide (**3a**) and β-alanine fenoprofenamide (**3b**) was performed in acetone/water solution, with benzotriazolide/amino acid ratio 1:1, in the presence of TEA (Zovko et al., 2001). Benzotriazole activated fenoprofen **2** was also the starting compound in the synthesis of 2-aminoethyl fenoprofenamide (**3c**), while the nucleophilic component was ethylenediamine. In this reaction the excess of the amine was crucial to avoid the formation of bis-fenoprofen ethylenediamide. The structure of **3c** was char-



Scheme 1.

acterised in full by IR, ^{1}H and ^{13}C NMR spectra (Fig. 1).

In the following reaction step, amino acid fenoprofen derivatives **3a** and **3b** were transformed to the benzotriazolides **6a** and **6b**, respectively, by reaction of the corresponding **3** with chloride **1** (Scheme 2). The reactions were performed in dry toluene, with the reactants ratio 1:1, in the presence of TEA as HCl acceptor. Spectral analyses of **6a** and **6b** were consistent with the assigned structures. IR spectra showed benzotriazolide carbonyl absorption maxima at 1750 and 1591, in addition to amide carbonyl absorptions at 1641 and 1549 cm^{-1}. Fig. 2 shows chemical structure and atom enumeration of benzotriazolides **2, 6a** and **6b**, while Table 1 presents their ^{13}C NMR chemical shifts and assignments compared with fenoprofen data.

Compounds **2, 6a**,**b** and **3c** proved to be useful in synthesis of polymer-fenoprofen conjugates. As polymer components, two polyhydroxy aspartamide-type polymers PHEA (7) and PHPA (4) were chosen, due to their reactive hydroxy functionalities, hydrosolubity and biocompatibility. The starting polymers **7** and **4** were prepared from PSI (**10**) and the corresponding aminoalcohol, according the procedures previously published (Neri et al., 1973; Jakopović et al. 1996; Lovrek et al., 2000) (Scheme 3). The completion of aminolysis was checked my IR spectroscopy (absence of succinimide absorption at 1715 cm^{-1}). Average molecular masses of PHEA and PHPA were 61000 (Lovrek et al., 2000). The syntheses of the conjugates **5, 8** and **9** were essentially esterification of polyhydroxy polymers **7** and **4** by the azole activated fenoprofen derivatives **2** and **6**. The reactions were performed in DMF, at room temperature, in the presence of TEA. The prepared conjugates were assigned as PHPA-Fen (**5**), PHEA-Gly-Fen (**8a**), PHEA-β-Ala-Fen (**8b**), PHPA-Gly-Fen (**9a**) and PHPA-β-Ala-Fen (**9b**).

The conjugate **11** (PAHA-Fen) was prepared by partial aminolysis of PSI with aminoamide **3c**, followed by aminolysis of the remaining succinimide units by means of 2-hydroxyethylamine (Scheme 4).

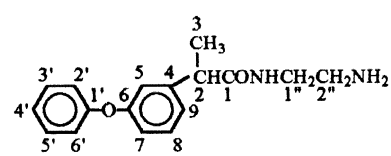
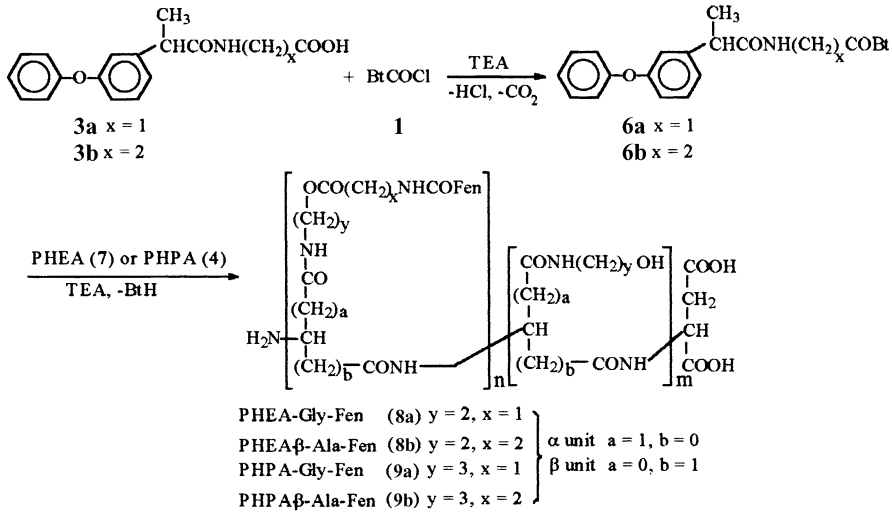


Fig. 1. Chemical structure and atom enumeration of 2-aminoethyl fenoprofenamide (**3c**).



Scheme 2.

Purification of **5, 8, 9** and **11** from the unbound fenoprofen derivatives, benzotriazole by-product or amine excess was successfully performed by triturating of the crude conjugates with solvents which selectively dissolved impurities, but not the conjugates (TLC control).

The synthesised polymer-drug conjugates differed in type of covalent bounding, type and/or length of spacer and drug-loading. In conjugate **5** fenoprofen was linked by an ester bond to hydroxy substituents of PHPA, through propyl spacer present in polymer itself. In the conjugates **8** and **9** fenoprofen was linked by an amide bond to glycine (**8a**, **9a**) or β-alanine spacer (**8b**, **9b**), while the amino acid component was bound to hydroxyalkyl PHEA and PHPA residues by an ester linkage. In **11** both linkages, i.e. polymer-spacer and spacer-fenoprofen, were of amide type. This conjugate is a derivative of 2-amino- and 2-hydroxyethylaspartamide copolymer (PAHA), which has been also described and used as a drug carrier (Giammona et al., 1989; Zorc et al., 1995).

Solubility of the synthesised conjugates depended on the drug loading. To assure hydrosolubility, less than one third of the available hydroxy groups in PHEA and PHPA was substituted by fenoprofen residue.

The drug loading in polymer-fenoprofen conjugates was estimated by UV-spectroscopy using the molar absorption coefficient for fenoprofen $\varepsilon_{272} = 1748\ dm^3\ mol^{-1}\ cm^{-1}$ (in H_2O, $c = 2.25 \times 10^{-4}$ mol l^{-1}). The percentage of fenoprofen was in the range from 8.7 to 20.4% (Table 2). The drug loading was depended on the molar ratio of the reactants **2, 6a**,**b** or **3c** and monomer units of the corresponding polymer **4, 7** or **10**, but was not strictly stoichoimetric. The values of experimentally determined drug loading were always less than the expected ones, due to incomplete coupling reactions.

The proof that fenoprofen was covalently bound in the synthesised polymer-drug conjugates could be found in the UV-spectra. All prepared

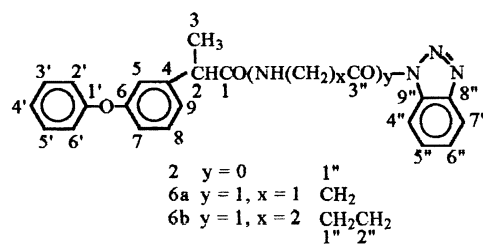
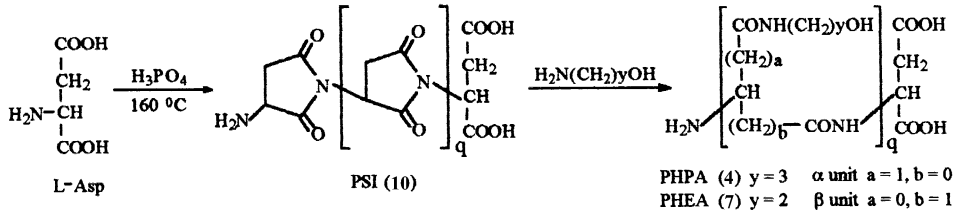


Fig. 2. Chemical structure and atom enumeration of fenoprofen benzotriazolide (**2**), benzotriazolide of glycine fenoprofenamide (**6a**) and benzotriazolide of β-alanine fenoprofenamide (**6b**).



Scheme 3.

conjugates absorbed UV-light in the same absorption ranges as fenoprofen, whereas PHEA, PHPA and PAHA themselves had no UV-absorption at these wavelengths. IR spectra of the conjugates bearing ester functionalities showed additional carbonyl absorption peaks at 1732–1738, besides strong amide carbonyl absorptions at 1653 (amide I) and 1540 (amide II). The absence of non-conjugated drug was confirmed by TLC using methanol, dichloromethane/methanol (9.5:0.5) and cyclohexane/ethyl acetate (1:1) solvent systems in which polymer derivatives remained at start and fenoprofen, benzotriazolides **2, 6a**,**b** or aminoamide **3c** moved with the mobile phase. A portion of conjugate **5** was dissolved in water and dyalised in order to compare the fenoprofen loading before and after dialysis. The fact that the drug loading was the same proved that all fenoprofen was chemically bound. The absence of the free fenoprofen in the prepared conjugates was also confirmed by DSC-thermograms. The conjugates melted between 250 and 270 °C while fenoprofen melted at 118–123 °C.

Table 1
^{13}C NMR data of fenoprofen, fenoprofen benzotriazolide (**2**), benzotriazolide of glycine fenoprofenamide (**6a**) and benzotriazolide of β-alanine fenoprofenamide (**6b**)

C atom	Fenoprofen	Chemical shifts in DMSO-d_6 (δ ppm)		
		2	**6a**	**6b**
1	175.67	172.80	174.15	173.32
2	45.08	44.59	44.60	44.94
3	18.80	18.61	18.73	18.48
4	143.99	145.50	144.13	144.40
5	117.98	118.09	116.87	116.58
6	156.97	157.02	156.72	156.63
7	116.91	117.20	117.96	117.63
8	130.13	130.52	129.89	130.77
9	122.77	122.64	122.68	122.34
1′	156.78	156.25	156.56	156.51
2′	118.91	118.81	118.63	118.62
3′	130.24	130.10	130.09	130.07
4′	123.68	123.72	123.44	123.44
5′	130.24	130.10	130.09	130.07
6′	118.91	118.81	118.63	118.62
1″	–	–	42.74	35.34
2″	–	–	–	34.52
3″	–	–	168.72	170.84
4″	–	114.07	114.99	114.10
5″	–	120.14	120.21	120.08
6″	–	126.63	126.66	126.45
7″	–	130.99	128.97	130.77
8″	–	141.89	145.34	145.55
9″	–	130.78	138.83	130.63

On the basis of previous results for the similar PHEA-NSAID conjugate (Giammona et al., 1991; Zorc et al., 1993; Zorc and Butula 1994) it could be speculated that all conjugates would undergo chemical hydrolysis in more or less comparable rates, but would differ in enzymatic cleavage. Prolonged distance between main chain and drug component in conjugates **8** and **9** could enable easier approach of enzymes which would theoretically cleave the covalent bonds between drug and spacer, polymer backbone and spacer, or both, but this assumption still remain to be confirmed.

Acknowledgements

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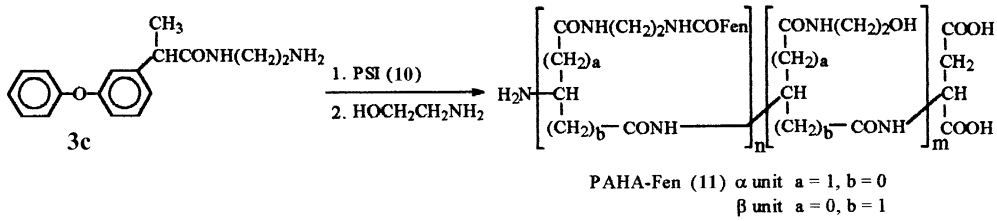

Scheme 4.

Table 2
Preparation and characterisation of polymer-fenoprofen conjugates

Conjugates	Reactants		Yield (%)	Drug loading (%)	UV: λ_{max} 272 nm	
					γ (μg ml^{-1})	A
PHPA-Fen (**5**)	PHPA (**4**)	**2**	42	20.1	130.4[a,b]	0.423
PHEA-Gly-Fen (**8a**)	PHEA (**7**)	**6a**	64	8.9	360.0[c]	0.514
PHEA-β-Ala-Fen (**8b**)	PHEA (**7**)	**6b**	46	20.4	109.6[c]	0.360
PHPA-Gly-Fen (**9a**)	PHPA (**4**)	**6a**	60	9.4	392.0[c]	0.591
PHPA-β-Ala-Fen (**9b**)	PHPA (**4**)	**6b**	75	12.8	250.0[c]	0.516
PAHA-Fen (**11**)	PSI (**10**)	**3c**	51	8.7	360.0[a]	0.505

[a] Solvent: H_2O.
[b] λ_{max} 279 nm.
[c] Solvent: ethanol/H_2O (2.5:1).

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