Supplementary material

Selective nucleoside triphosphate diphosphohydrolase-2 (NTPDase2) inhibitors: nucleotide mimetics derived from uridine-5'-carboxamide

*Andreas Brunschweiger[§], Jamshed Iqbal[§], Frank Umbach,[§] Anja B. Scheiff[§], Mercedes N. Munkonda[#], Jean Sévigny[#], Aileen F. Knowles[‡] and Christa E. Müller[§]**

[§]Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany, [#] Centre de Recherche en Rhumatologie et Immunologie, Centre Hospitalier Universitaire de Québec, Université Laval, Québec, QC, Canada, [‡]Department of Chemistry and Biochemistry, San Diego State University, San Diego, CA 92182-1030, USA

Table of Contents

Table 1. Elemental analyses for compounds **19-23**

Compd.	Formula	Calculated			Found		
		C	H	N	C	H	N
19a	$C_{22}H_{29}N_4O_{10}P \cdot 4.25\ H_2O$	42.82	6.08	9.08	42.67	5.60	8.82
19b	$C_{23}H_{31}N_4O_{10}P \cdot 4\ H_2O$	44.08	6.23	8.95	43.92	5.99	8.81
19c	$C_{24}H_{33}N_4O_{10}P \cdot 6\ H_2O$	41.50	6.47	8.06	41.15	6.10	7.89
20a	$C_{16}H_{25}N_4O_{10}P \cdot 2.75\ H_2O$	38.37	6.01	10.85	38.35	5.96	10.54
20b	$C_{17}H_{27}N_4O_{10}P \cdot 1.5\ H_2O$	40.39	5.94	11.08	40.17	5.60	11.25
20c	$C_{18}H_{29}N_4O_{10}P \cdot H_2O$	42.35	6.07	10.98	42.17	5.88	10.79
21a	$C_{17}H_{27}N_4O_{10}P$	42.68	5.69	11.71	42.54	5.76	11.56
22a	$C_{23}H_{30}N_7O_8P \cdot 2.75\ H_2O$	45.06	5.80	16.00	45.53	6.06	15.60
22b	$C_{24}H_{32}N_7O_8P \cdot 2\ H_2O$	46.98	5.87	15.98	47.04	6.39	15.65
22c	$C_{25}H_{34}N_7O_8P \cdot 2.5\ H_2O$	47.59	6.17	15.22	47.98	6.46	15.14
23a	$C_{17}H_{26}N_7O_8P \cdot H_2O$	40.39	5.55	19.41	40.54	5.65	19.08
23b	$C_{18}H_{28}N_7O_8P \cdot 2.75\ H_2O$	39.23	6.09	17.80	39.65	5.79	17.15
23c	$C_{19}H_{30}N_7O_8P \cdot 3\ H_2O$	40.07	6.33	17.22	40.31	6.57	16.83

Yields and analytical data for compounds 7, 8, 16-18 and 19-23

7a: **2',3'-*p*-Methoxybenzylideneadenosine**. Yield: 94 %; mp: 215 °C (lit: 215 °C)[1]. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.36 (2 x s, 1H, H-2), 8.15 (2 x s, 1H, H-8), 7.50 and 7.48 (2 x dd, 2H, ^3J = 8.85 Hz and ^4J = 2.85 Hz, 2 x H_{ortho}, *p*-methoxybenzylidene), 7.31 (br s, 2H, 6-NH_2), 6.98 and 6.95 (2 x dd, 2H, ^3J = 8.85 Hz and ^4J = 2.80 Hz, 2 x H_{meta}, *p*-methoxybenzylidene), 6.26 (2 x d, 1H, ^3J = 3.15 Hz, H-1'), 6.18 and 5.97 (1H, 2 x s, Ph-CH), 5.47 and 5.45 (2 x dd, 1H, ^3J = 3.15 Hz and ^3J = 6.30 Hz, H-2'), 5.22 and 5.11 (2 x t, 1H, ^3J = 5.70 Hz, 5'-OH), 5.06 and 5.05 (2 x dd, 1H, ^3J = 6.60 Hz and ^3J = 2.85 Hz, H-3'), 4.36 and 4.26 (dt and pseudo-q, ^3J = 5.05 Hz and ^3J = 2.55 Hz, H-4'), 3.78 and 3.76 (2 x s, 3H, O-CH_3), 3.64 – 3.51 (m, 2H, ^3J = 5.00 Hz and ^3J = 3.15 Hz, 2 x H-5'). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 160.5 and 160.4 (C_{para}, *p*-methoxybenzylidene), 156.3 (C-6), 152.8 (C-2), 149.1 and 149.0 (C-4), 140.0 and 139.8 (C-8), 128.6 and 128.5 (2 x C_{meta}, *p*-methoxybenzylidene), 128.4 and 128.3 (C_{ipso}, *p*-methoxybenzylidene), 119.3 and 119.2 (C-5), 113.9 and 113.8 (2 x C_{ortho}, *p*-methoxybenzylidene), 106.7 and 103.0 (Ph-CH), 89.6 and 88.1 (C-1'), 86.4 and 84.6 (C-4'), 83.8 and 83.0 (C-2'), 82.7 and 80.6 (C-3'), 61.7 (C-5'), 55.3 (O-CH_3). Double peaks are due to the formation of diastereomers.

7b: **2',3'-*p*-Methoxybenzylideneuridine**. Yield: 93 %; mp: 213 °C (lit: 213 °C)[2]. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 11.38 (1H, br, s, NH), 7.85 and 7.76 (1 H, 2 x d, ^3J = 7.90 Hz, H-6), 7.44 and 7.39 (2H, 2 x dd, ^3J = 6.95 Hz and ^4J = 2.25 Hz, 2 x H_{ortho}, *p*-methoxybenzylidene), 6.97 and 6.95 (2H, 2 x dd, ^3J = 6.65 Hz and ^4J = 2.20 Hz, 2 x H_{meta}, *p*-methoxybenzylidene), 6.08 and 5.94 (1H, 2 x s, Ph-CH), 5.94 (1H, 2 x d, ^3J = 2.90 Hz, H-1'), 5.64 (1H, pseudo-t, ^3J = 7.55 Hz, H-5), 5.09 and 5.05 (1H, 2 x t, ^3J = 5.35 Hz, 5'-OH), 5.01 and 4.98 (1H, 2 x dd, ^3J = 6.60 Hz and ^3J = 2.85 Hz, H-2'), 4.86 and 4.82 (1H, 2 x dd, ^3J = 6.60 Hz and ^3J = 4.40 Hz, H-3'), 4.23 and 4.15 (1H, 2 x dt, ^3J = 4.80 Hz and ^3J = 3.15 Hz, H-4'), 3.75 and 3.77 (3H, 2 x s, O-CH_3), 3.67 – 3.61 (2H, m, ^3J = 5.35 Hz and ^3J = 3.20 Hz, 2 x H-5'). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 163.3 (C-4), 160.5 (C_{para} , *p*-methoxybenzylidene), 150.5 (C-2), 142.2 (C-6), 128.5 (2 x C_{meta}, *p*-methoxybenzylidene), 128.4 (C_{ipso}, *p*-methoxybenzylidene), 113.9 and 113.8 (2 x C_{ortho}, *p*-methoxybenzylidene), 106.6 (Ph-CH), 103.6 (C-5), 91.4 and 90.5 (C-1'), 86.5 (C-4'), 84.3 and 84.1 (C-2'), 83.0 and 81.8 (C-3'), 61.5 (C-5'), 55.4 and 55.3 (O-CH_3). Double peaks are due to the formation of diastereomers.

8a: **2',3'-*p*-Methoxybenzylideneadenosine-5'-carboxylic acid**. Yield: 71 %; mp: 241 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.70 (br s, 1H, COOH), 8.27 (2 x s, 1H, H-2), 8.08 (2 x s, 1H, H-8), 7.48 and 7.45 (2 x dd, 2H, ^{3}J = 8.85 Hz and ^{4}J = 2.85 Hz, 2 x H_{ortho}, *p*-methoxybenzylidene), 7.27 (br s, 2H, 6-NH_2), 6.98 and 6.96 (2 x dd, 2H, ^{3}J = 8.85 Hz and ^{4}J = 2.80 Hz, 2 x H_{meta}, *p*-methoxybenzylidene), 6.47 and 6.44 (2 x s, 1H, H-1'), 6.12 and 5.93 (1H, 2 x s, Ph-CH), 5.74 and 5.62 (2 x dd, 1H, ^{3}J = 6.30 Hz, ^{3}J = 2.55 Hz, H-3'), 5.59 and 5.48 (2 x d, 1H, ^{3}J = 6.30 Hz, H-2'), 4.87 and 4.85 (2 x d, ^{3}J = 2.50 Hz and ^{3}J = 1.55 Hz, H-4'), 3.78 and 3.77 (2 x s, 3H, O-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.9 and 170.7 (COOH), 160.6 and 160.5 (C_{para}, *p*-methoxybenzylidene), 156.2 (C-6), 125.6 and 152.5 (C-2), 149.3 (C-4), 140.8 and 140.6 (C-8), 128.7 (2 x C_{meta}, *p*-methoxybenzylidene), 128.1 and 128.0 (C_{ipso}, *p*-methoxybenzylidene), 118.9 (C-5), 113.9 (2 x C_{ortho}, *p*-methoxybenzylidene), 105.8 and 103.6 (Ph-CH), 89.5 and 89.4 (C-1'), 85.7 and 84.5 (C-4'), 84.2 and 83.9 (C-2'), 83.8 and 82.7 (C-3'), 55.4 (O-CH_3). Double peaks are due to the formation of diastereomers.

8b: **2',3'-*p*-methoxybenzylideneuridine-5'-carboxylic acid**. Yield: 78 %; mp: 196 °C. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 12.70 (br s, 1H, COOH), 11.32 (1H, br, s, NH), 7.82 and 7.74 (1 H, 2 x d, ^{3}J = 7.85 Hz, H-6), 7.44 and 7.39 (2H, 2 x dd, ^{3}J = 6.85 Hz and ^{4}J = 1.85 Hz, 2 x H_{ortho}, *p*-methoxybenzylidene), 6.97 and 6.95 (2H, 2 x dd, ^{3}J = 6.60 Hz and ^{4}J = 3.15 Hz, 2 x H_{meta}, *p*-methoxybenzylidene), 5.98 and 5.83 (1H, 2 x s, Ph-CH), 5.96 (1H, s, H-1'), 5.64 (1H, d, ^{3}J = 7.85 Hz, H-5), 5.38 and 5.27 (1H, dd (5.38) and br s (5.19), ^{3}J = 6.00 Hz, ^{3}J = 2.55 Hz, H-3'), 5.27 and 5.19 (1H, br s (5.27) and d (5.19), ^{3}J = 5.70 Hz, H-2'), 4.74 and 4.73 (1H, 2 x d, ^{3}J = 2.50 Hz and ^{3}J = 0.95 Hz, H-4'), 3.67 and 3.77 (3H, 2 x s, O-CH_3). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 171.0 and 170.9 (COOH), 163.6 (C-4), 160.5 and 160.4 (C_{para}, *p*-methoxybenzylidene), 151.1 and 151.0 (C-2), 145.2 and 144.9 (C-6), 128.5 (2 x C_{meta}, *p*-methoxybenzylidene), 128.1 (C_{ipso}, *p*-methoxybenzylidene), 114.2 and 113.8 (2 x C_{ortho}, *p*-methoxybenzylidene), 105.3 and 103.0 (Ph-CH), 101.6 (C-5), 95.8 and 95.6 (C-1'), 87.0 and 85.1 (C-4'), 84.8 and 84.5 (C-2'), 84.2 and 82.9 (C-3'), 55.5 (O-CH_3). Double peaks are due to the formation of diastereomers.

16b: *p*-(2-Aminoethylcarboxamido)benzylphosphonic acid diethyl ester hydrochloride. Yield over two steps: 3.2 g, 87 %; 198 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.20 (br s, 1H, O=C-NH), 7.93 (br s, 3H, NH_3^+), 7.52 (d, 2H, ^{3}J = 8.50 Hz, 2 x CH_{ortho}, benzyl phosphonate), 7.19 (dd, 2H, ^{3}J = 8.50 Hz and ^{4}J = 2.50 Hz, 2 x CH_{meta}, benzyl phosphonate), 3.95 – 3.90 (2 x q, 4H, 2 x O-CH_2), 3.13 (d, 2H, $^{2}J_{H,P}$ = 21.45 Hz, CH_2-P, benzyl phosphonate), 3.07 (br s, 2H, N-CH_2, ethylcarboxamide), 2.71 (t, 2H, ^{3}J = 6.95 Hz, O=C-CH_2, ethylcarboxamide), 1.21 (t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 168.3 (C=O), 137.5 (C_{para}, benzyl phosphonate), 130.2 and 130.1 (2 x C_{ortho}, benzyl phosphonate), 127.1 (d, $^{2}J_{C,P}$ = 8.2 Hz, C_{ipso}, benzyl phosphonate), 119.2 and 119.1 (2 x C_{meta}, benzyl phosphonate), 61.5 (2 x O-CH_2), 35.0 (N-CH_2, ethylcarboxamide), 33.2 (O=C-CH_2, ethylcarboxamide), 31.8 (d, $^{1}J_{C,P}$ = 134.8 Hz, CH_2-P, benzyl phosphonate), 16.4 and 16.3 (2 x CH_3). ^{31}P-NMR (202 MHz, DMSO-d_6) δ 26.3.

16c: *p*-(3-Aminopropylcarboxamido)benzylphosphonic acid diethyl ester hydrochloride. Yield over two steps: 3.5 g, 93 %; mp: 154 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.11 (br s, 1H, O=C-NH), 8.00 (br s, 3H, NH_3^+), 7.53 (d, 2H, ^{3}J = 8.55 Hz, 2 x CH_{ortho}, benzyl phosphonate), 7.17 (dd, 2H, ^{3}J = 8.50 Hz and ^{4}J = 2.20 Hz, 2 x CH_{meta}, benzyl phosphonate), 3.95 – 3.89 (2 x q, 4H, 2 x O-CH_2), 3.36 (m, 2H, ^{3}J = 7.25 Hz, N-CH_2, propylcarboxamide), 3.13 (d, 2H, $^{2}J_{H,P}$ = 21.10 Hz, CH_2-P, benzyl phosphonate), 2.42 (t, 2H, ^{3}J = 7.25 Hz, O=C-CH_2, propylcarboxamide), 1.84 (m, 2H, ^{3}J = 7.60 Hz and ^{3}J = 7.25 Hz, CH_2, propylcarboxamide), 1.23 (2 x t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.3 (C=O), 137.8 (C_{para}, benzyl phosphonate), 130.1 and 130.0 (2 x C_{ortho}, benzyl phosphonate), 126.4 (d, $^{2}J_{C,P}$ = 8.9 Hz, C_{ipso}, benzyl phosphonate), 119.1 (2 x C_{meta}, benzyl phosphonate), 61.5 and 61.4 (2 x O-CH_2), 38.4 (N-CH_2, propylcarboxamide), 33.1 (O=C-CH_2, propylcarboxamide), 31.8 (d, $^{1}J_{C,P}$ = 134.8

Hz, CH_2-P, benzyl phosphonate), 23.2 (CH_2, propylcarboxamide), 16.4 and 16.3 (2 x CH_3). ^{31}P-NMR (202 MHz, DMSO-d_6) δ 26.3.

17a: Aminomethylcarboxamidomethylphosphonic acid diethyl ester hydrochloride. Yield over two steps: 2.2 g, 86 %; mp: 153 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.79 (t, 1H, ^3J = 5.65 Hz, O=C-NH), 8.23 (br s, 3H, NH_3^+), 4.06 – 4.00 (2 x q, 4H, 2 x O-CH_2), 3.61 (dd, 2H, ^2J = 12.00 Hz and $^2J_{H,P}$ = 17.95 Hz, CH_2-P, methyl phosphonate), 3.56 (s, 2H, N-CH_2, methylcarboxamide), 1.22 (t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 166.2 (C=O), 62.1 and 62.0 (2 x O-CH_2), N-CH_2 (methylcarboxamide) not detectable, under solvent peak at 45 ppm, 34.7 (d, $^1J_{C,P}$ = 154.4 Hz, CH_2-P, methyl phosphonate), 16.4 and 16.3 (2 x CH_3). ^{31}P-NMR (202 MHz, DMSO-d_6) δ 22.5.

17b: 2-Aminoethylcarboxamidomethylphosphonic acid diethyl ester hydrochloride. Yield over two steps: 1.8 g, 66 %; mp: 138 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.31 (t,1H, ^3J = 5.65 Hz, O=C-NH), 8.01 (br s, 3H, NH_3^+), 4.04 – 3.93 (2 x q, 4H, 2 x O-CH_2), 3.74 (dd, 2H, ^2J = 11.65 Hz and $^2J_{H,P}$ = 17.65 Hz, CH_2-P, methyl phosphonate), 3.51 (m, 2H, ^3J = 6.65 Hz, N-CH_2, ethylcarboxamide), 2.53 (m, 2H, ^3J = 6.60 Hz, O=C-$\underline{CH_2}$, ethylcarboxamide), 1.27 (2 x t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 169.4 (C=O), 61.9 and 61.2 (2 x O-CH_2), 35.5 (N-CH_2, ethylcarboxamide), 35.2 (d, $^1J_{C,P}$ = 137.9 Hz, CH_2-P), 33.9 (O=C-$\underline{CH_2}$, ethylcarboxamide), 16.34 (2 x CH_3). ^{31}P-NMR (202 MHz, DMSO-d_6), δ 28.8.

17c: 3-Aminopropylcarboxamidomethylphosphonic acid diethyl ester hydrochloride. Yield over two steps: 2.4 g, 84 %; mp: 104 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.29 (t, 1H, ^3J = 6.00 Hz, O=C-NH), 7.91 (br s, 3H, NH_3^+), 4.03 – 3.97 (2 x q, 4H, 2 x O-CH_2), 3.55 – 3.32 (dd, 2H, ^2J = 12.30 Hz and $^2J_{H,P}$ = 18.30 Hz, CH_2-P, methyl phosphonate), 2.79 – 2.72 (m, 2H, ^3J = 6.30 Hz, N-CH_2, propylcarboxamide), 2.22 (t, 2H, ^3J = 6.95 Hz, O=C-$\underline{CH_2}$, propylcarboxamide), 1.76 (tt, 2H, ^3J = 5.70 Hz and ^3J = 6.95 Hz, CH_2, propylcarboxamide), 1.21 (t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 171.7 (C=O), 62.1 (2 x O-CH_2), 38.8 (N-CH_2, propylcarboxamide), 34.3 (d, $^1J_{C,P}$ = 154.2 Hz, CH_2-P, methyl phosphonate), 32.2 (O=C-$\underline{CH_2}$, propylcarboxamide), 23.7 (CH_2, propylcarboxamide), 16.4 and 16.3 (2 x CH_3). ^{31}P-NMR (202 MHz, DMSO-d_6) δ 23.5.

18a: 2-(Aminomethylcarboxamido)ethylphosphonic acid diethyl ester hydrochloride. Yield over two steps: 1.9 g, 69 %; mp: 103 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.55 (t, 1H, ^3J = 5.65 Hz, CONH), 8.12 (br s, 3H, NH_3^+), 4.02 – 3.98 (2 x q, 4H, 2 x O-CH_2), 3.49 (s, 2H, N-CH_2, methylcarboxamide), 3.30 – 3.18 (m, 2H, ^3J = 7.55 Hz and ^3J = 5.45 Hz and $^3J_{H,P}$ = 12.95 Hz, N-CH_2, ethyl phosphonate), 1.97 – 1.91 (m, 2H, ^3J = 7.55 Hz and $^2J_{H,P}$ = 18.35 Hz, CH_2-P, ethyl phosphonate), 1.23 (t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 165.8 (C=O), 61.3 (2 x O-CH_2), N-CH_2 (methylcarboxamide) not detectable, under solvent peak at 42 ppm, 33.3 (d, $^2J_{C,P}$ = 14.9 Hz, N-CH_2, ethyl phosphonate), 25.3 (d, $^1J_{C,P}$ = 135.8 Hz, CH_2-P, ethyl phosphonate), 16.4 (2 x CH_3). ^{31}P-NMR (202 MHz, DMSO-d_6) δ 28.4.

19b: **4-[3-((*2S*,*3R*,*4S*,*5R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido)propylamido]benzylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: HBTU; yield over two steps: 350 mg, 63 %; mp: 255 °C. ^{1}H-NMR (500 MHz, MeOD) δ 8.08 (d, 1H, ^3J= 8.20 Hz, H-6), 7.55 (d, 2H, ^3J = 7.90 Hz, 2 x CH_{ortho}, benzyl phosphonate), 7.29 (dd, 2H, ^3J = 8.50 Hz and ^4J = 2.85 Hz, 2 x CH_{meta}, benzyl phosphonate), 5.92 (d, 1H, ^3J = 6.30 Hz, H-1'), 5.72 (d, 1H, ^3J = 7.90 Hz, H-5), 4.41 (dd, 1H, ^3J = 5.05 Hz and ^3J = 6.30 Hz, H-2'), 4.40 (d, 1H, ^{3} = 2.85, H-4'), 4.28 (dd, 1H, ^3J = 5.05 Hz and ^2J = 2.85 Hz, H-3'), 4.10 – 4.03 (2 x q, 4H, 2 x O-CH_2), 3.69 – 3.56 (m, 2H, ^3J = 6.30 Hz, N-CH_2, propylamide), 3.23 (d, 2H, $^2J_{H,P}$ = 21.45 Hz, CH_2-P, benzyl phosphonate), 2.65 (m, 2H, ^3J = 6.30 Hz, O=C-$\underline{CH_2}$, propylamide), 1.29 (t, 6H, 2 x CH_3). ^{13}C-NMR

(125 MHz, MeOD) δ 172.6 (C=O), 172.2 (C=O), 166.3 (C-4), 152.9 (C-2), 144.3 (C-6), 139.1 (C_{para}, benzyl phosphonate), 131.7 (2 x CH_{ortho}, benzyl phosphonate), 128.6 (d, $^{2}J_{C,P}$ = 9.4 Hz, C_{ipso}, benzyl phosphonate), 121.5 (2 x CH_{meta}, benzyl phosphonate), 103.4 (C-5), 92.4 (C-1'), 85.4 (C-4'), 74.9 (C-2'), 74.1 (C-3'), 64.1 and 64.0 (2 x O-CH_2), 36.6 (N-CH_2, propylamide), 37.1 (O=C-$\underline{CH_2}$, propylamide), 33.4 (d, $^{1}J_{C,P}$ = 137.6 Hz, CH_2-P, benzyl phosphonate), 16.9 and 16.8 (2 x CH_3). ^{31}P-NMR (202 MHz, MeOD) δ 26.8. LC/ESI-MS: negative mode 553.3 ($[M-H]^-$), positive mode 555.3 ($[M+H]^+$).

19c: **4-[4-((*2S*,*3R*,*4S*,*5R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido)butylamido]benzylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: HBTU; yield over two steps: 390 mg, 69 %; mp: 248 °C. ^{1}H-NMR (500 MHz, MeOD) δ 8.11 (d, 1H, ^{3}J = 8.20 Hz, H-6), 7.55 (d, 2H, ^{3}J = 7.90 Hz, 2 x CH_{ortho}, benzyl phosphonate), 7.28 (dd, 2H, ^{3}J = 8.50 Hz and ^{4}J = 2.85 Hz, 2 x CH_{meta}, benzyl phosphonate), 5.86 (d, 1H, ^{3}J = 6.30 Hz, H-1'), 5.75 (d, 1H, ^{3}J = 7.90 Hz, H-5), 4.45 (dd, 1H, ^{3}J = 5.05 Hz and ^{3}J = 6.30 Hz, H-2'), 4.39 (d, 1H, ^{3}J = 2.85, H-4'), 4.27 (dd, 1H, ^{3}J = 5.05 Hz and ^{2}J = 2.85 Hz, H-3'), 4.09 – 4.03 (2 x q, 4H, 2 x O-CH_2), 3.38 (t, partly below solvent peak, 2H, ^{3}J = 7.25 Hz, N-CH_2, butylamide), 3.23 (d, 2H, $^{2}J_{H,P}$ = 21.45 Hz, CH_2-P, benzyl phosphonate), 2.45 (t, 2H, ^{3}J = 7.25 Hz, O=C-$\underline{CH_2}$, butylamide), 1.95 (tt, 2H, ^{3}J = 7.25 Hz, CH_2, butylamide), 1.29 (t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, MeOD) δ 174.0 (C=O), 172.7 (C=O), 166.3 (C-4), 152.9 (C-2), 144.6 (C-6), 139.2 (C_{para}, benzyl phosphonate), 131.6 (2 x CH_{ortho}, benzyl phosphonate), 128.5 (d, $^{2}J_{C,P}$ = 9.4 Hz, C_{ipso}, benzyl phosphonate), 121.4 (2 x CH_{meta}, benzyl phosphonate), 103.3 (C-5), 93.1 (C-1'), 85.4 (C-4'), 74.8 (C-2'), 74.1 (C-3'), 64.0 (2 x O-CH_2), 40.1 (N-CH_2, butylamide), 35.5 (O=C-$\underline{CH_2}$, butylamide), 33.4 (d, $^{1}J_{C,P}$ = 137.6 Hz, CH_2-P, benzyl phosphonate), 26.6 (CH_2, butylamide), 16.9 and 16.8 (2 x CH_3). ^{31}P-NMR (202 MHz, MeOD) δ 26.8. LC/ESI-MS: negative mode 567.3 ($[M-H]^-$), positive mode 569.2 ($[M+H]^+$).

20a: **2-[(*2S*,*3R*,*4S*,*5R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido]ethylamidomethylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: HCTU; yield over two steps: 190 mg, 41 %; mp: 105 °C. ^{1}H-NMR (500 MHz, MeOD) δ 8.10 (d, 1H, ^{3}J = 7.85 Hz, H-6), 5.91 (d, 1H, ^{3}J = 5.95 Hz, H-1'), 5.77 (d, 1H, ^{3}J = 7.85 Hz, H-5), 4.50 (dd, 1H, ^{3}J = 5.35 Hz and ^{3}J = 5.70 Hz, H-2'), 4.48 (d, 1H, ^{3}J = 2.85 Hz, H-4'), 4.44 (dd, 1H, ^{3}J = 5.0 Hz and ^{3}J = 3.15 Hz, H-3'), 4.22 – 4.15 (2 x q, 4H, 2 x O-CH_2), 4.10 – 3.83 (AB-system with A d and B d, 2H, ^{2}J = 17.00 Hz, N-CH_2, ethylamide), 3.84 – 3.72 (AB-system with A dd and B dd, 2H, $^{2}J_{H,P}$ = 11.65 Hz and ^{2}J = 15.75 Hz, N-CH_2, methyl phosphonate), 1.35 (2 x t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, MeOD) δ 173.2 (C=O), 171.6 (C=O), 166.4 (C-4), 153.1 (C-2), 144.9 (C-6), 103.5 (C-5), 93.9 (C-1'), 85.7 (C-4'), 74.9 (C-2'), 74.2 (C-3'), 64.5 (2 x O-CH_2), 43.4 (N-CH_2, ethylamide), 35.7 (d, $^{1}J_{C,P}$ = 157.6 Hz, CH_2-P, methyl phosphonate), 17.0 (2 x CH_3). ^{31}P-NMR (202 MHz, MeOD) δ 22.1. LC/ESI-MS: negative mode 463.1 ($[M-H]^-$), positive mode 465.3 ($[M+H]^+$).

20b: **3-[(*2S*,*3R*,*4S*,*5R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido]propylamidomethylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: PyBOP; yield over two steps: 170 mg, 36 %; mp: 94 °C. ^{1}H-NMR (500 MHz, MeOD) δ 8.13 (d, 1H, ^{3}J = 8.20 Hz, H-6), 5.91 (d, 1H, ^{3}J = 6.00 Hz, H-1'), 5.79 (d, 1H, ^{3}J = 8.20 Hz, H-5), 4.44 (dd, 1H, ^{3}J = 5.05 Hz and ^{3}J = 5.95 Hz, H-2'), 4.38 (d, 1H, ^{3}J = 3.20 Hz, H-4'), 4.28 (dd, 1H, ^{3}J = 5.05 Hz and ^{3}J = 3.15 Hz, H-3'), 4.21 – 4.15 (2 x q, 4H, 2 x O-CH_2), 3.74 (d, 2H, $^{2}J_{H,P}$ = 11.65 Hz, CH_2-P, methyl phosphonate), 3.51 (m, 2H, ^{3}J = 6.65 Hz, N-CH_2, propylamide), 2.53 (m, 2H, ^{3}J = 6.60 Hz, O=C-$\underline{CH_2}$, propylamide), 1.37 (2 x t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, MeOD) δ 173.7 (C=O), 172.6 (C=O), 166.8 (C-4), 153.2 (C-2), 144.5 (C-6), 103.4 (C-5), 92.8 (C-1'), 85.4 (C-4'), 74.9 (C-2'), 74.0 (C-3'), 64.4 (2 x O-CH_2), 37.1 (N-CH_2, propylamide), 36.4 (O=C-$\underline{CH_2}$, propylamide), 35.7 (d, $^{1}J_{C,P}$ = 145.7 Hz, CH_2-P, methyl phosphonate), 17.0 (2 x CH_3). ^{31}P-NMR (202 MHz, MeOD) δ 24.6. LC/ESI-MS: negative mode 477.1 ($[M-H]^-$), positive mode 479.0 ($[M+H]^+$).

20c: **4-[(*2S*,*3R*,*4S*,*5R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido]butylamidomethylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: PyBOP; yield over two steps: 310 mg, 63 %; mp: 118 °C. ^{1}H-NMR (500 MHz, MeOD) δ 8.11 (d, 1H, ^{3}J = 7.85 Hz, H-6), 5.84 (d, 1H, ^{3}J = 6.00 Hz, H-1'), 5.78 (d, 1H, ^{3}J = 8.20 Hz, H-5), 4.50 (dd, 1H, ^{3}J = 5.05 Hz and ^{3}J = 5.95 Hz, H-2'), 4.39 (d, 1H, ^{3}J = 3.15 Hz, H-4'), 4.27 (dd, 1H, ^{3}J = 5.05 Hz and ^{3}J = 3.15 Hz, H-3'), 4.20 – 4.15 (2 x q, 4H, 2 x O-CH_2), 3.75 (d, 2H, $^{2}J_{H,P}$ = 11.65 Hz, CH_2-P, methyl phosphonate), 3.38 – 3.26 (m, partly below solvent peak, 2H, ^{3}J = 6.95 Hz, N-CH_2, butylamide), 2.34 (t, 2H, ^{3}J = 7.55 Hz, O=C-$\underline{CH_2}$, butylamide), 1.88 (tt, 2H, ^{3}J = 7.25 Hz and ^{3}J = 6.95 Hz, CH_2, butylamide), 1.36 (2 x t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, MeOD) δ 175.5 (C=O), 172.7 (C=O), 166.4 (C-4), 153.0 (C-2), 144.9 (C-6), 103.3 (C-5), 93.6 (C-1'), 85.5 (C-4'), 74.9 (C-2'), 73.9 (C-3'), 64.4 (2 x O-CH_2), 39.9 (N-CH_2, butylamide), 35.6 (d, $^{1}J_{C,P}$ = 156.8 Hz, CH_2-P, methyl phosphonate), 34.3 (O=C-$\underline{CH_2}$, butylamide), 26.8 (CH_2, butylamide), 17.0 (2 x CH_3). ^{31}P-NMR (202 MHz, MeOD) δ 24.6. LC/ESI-MS: negative mode 491.5 ($[M-H]^-$), positive mode 493.1 ($[M+H]^+$).

21a: **2-[2-((*2S*,*3R*,*4S*,*5R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido)ethylamido]ethylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: HCTU; yield over two steps: 228 mg, 48 %; mp: 131 °C. ^{1}H-NMR (500 MHz, MeOD) δ 8.12 (d, 1H, ^{3}J = 8.20 Hz, H-6), 5.93 (d, 1H, ^{3}J = 5.95 Hz, H-1'), 5.78 (d, 1H, ^{3}J = 8.20 Hz, H-5), 4.48 (dd, 1H, ^{3}J = 5.35 Hz and ^{3}J = 6.00 Hz, H-2'), 4.48 (d, 1H, ^{3}J = 3.15 Hz, H-4'), 4.40 (dd, 1H, ^{3}J = 5.05 Hz and ^{3}J = 3.20 Hz, H-3'), 4.19 – 4.12 (2 x q, 4H, 2 x O-CH_2), 4.01 - 3.83 (AB-system with A d and B d, 2H, $^{2}J_{A,B}$ = 16.70 Hz, N-CH_2, ethylamide), 3.50 (m, 2H, ^{3}J = 7.55 Hz and $^{3}J_{H,P}$ = 12.95 Hz, N-CH_2, ethyl phosphonate), 2.16 – 2.09 (m, 2H, ^{3}J = 7.55 and $^{2}J_{H,P}$ = 18.35 Hz, CH_2-P, ethyl phosphonate), 1.37 (t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, MeOD) δ 173.2 (C=O), 171.6 (C=O), 166.4 (C-4), 153.0 (C-2), 144.6 (C-6), 103.4 (C-5), 93.1 (C-1'), 85.4 (C-4'), 74.8 (C-2'), 74.1 (C-3'), 63.8 (2 x O-CH_2), 43.5 (N-CH_2, ethylamide), 34.9 (N-CH_2, ethyl phosphonate), 26.5 (d, $^{1}J_{C,P}$ = 138.3 Hz, CH_2-P, ethyl phosphonate), 17.0 (2 x CH_3). ^{31}P-NMR (202 MHz, MeOD) δ 28.8. LC/ESI-MS: negative mode 479.0 ($[M-H]^-$), positive mode 477.1 ($[M+H]^+$).

22a: **4-[2-((*2S*,*3R*,*4S*,*5R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido)ethylamido]benzylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: HCTU; yield over two steps: 228 mg, 68 %; mp: 146 °C. ^{1}H-NMR (500 MHz, MeOD), δ 8.42 (s, 1H, H-2), 8.24 (s, 1H, H-8), 7.57 (d, 2H, ^{3}J = 8.20 Hz, 2 x CH_{meta}, benzyl phosphonate), 7.30 (dd, 2H, ^{3}J = 8.80 Hz and ^{4}J = 2.50 Hz, 2 x CH_{ortho}, benzyl phosphonate), 6.14 (d, 1H, ^{3}J = 7.90 Hz, H-1'), 4.91 (dd, partly below solvent peak, 1H, ^{3}J = 7.55 Hz and ^{3}J = 4.75 Hz, H-2'), 4.62 (s, 1H, H-4'), 4.49 (dd, 1H, ^{3}J = 4.75 and ^{3}J = 1.60 Hz, H-3'), 4.29 - 4.10 (AB-system with A d and B d, 2H, ^{2}J = 16.40 Hz, N-CH_2, ethylamide), 4.09 – 4.04 (m, 4H, 2 x O-CH_2), 3.24 (d, 2H, $^{2}J_{H,P}$ = 21.45 Hz, CH_2-P, benzyl phosphonate), 1.29 (t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, MeOD) δ 173.4 (C=O), 169.5 (C=O), 157.9 (C-6), 154.2 (C-2), 150.6 (C-4), 142.8 (C-8), 138.9 (C_{para}, benzyl phosphonate), 131.7 (2 x CH_{ortho}, benzyl phosphonate), 128.7 (d, $^{2}J_{C,P}$ = 9.4 Hz, C_{ipso}, benzyl phosphonate), 121.5 (2 x CH_{meta}, benzyl phosphonate), 121.3 (C-5), 90.6 (C-1'), 86.7 (C-4'), 75.5 (C-2'), 73.9 (C-3'), 64.0 (2 x O-CH_2), 44.0 (N-CH_2, ethylamide), 33.4 (d, $^{1}J_{C,P}$ = 137.6 Hz, CH_2-P, benzyl phosphonate), 17.0 und 16.9 (2 x CH_3). ^{31}P-NMR (202 MHz, MeOD) δ 28.8. LC/ESI-MS: negative mode 562.3 ($[M-H]^-$), positive mode 564.3 ($[M+H]^+$).

22b: **4-[3-((*2S*,*3R*,*4S*,*5R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido)propylamido]benzylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: HBTU; yield over two steps: 317 mg, 55 %; mp: 111°C. ^{1}H-NMR (500 MHz, D_2O) δ 8.08 (s, 1H, H-2), 7.95 (s, 1H, H-8), 7.57 (d, 2H, ^{3}J = 8.20 Hz, 2 x CH_{meta}, benzyl phosphonate), 7.30 (dd, 2H, ^{3}J = 8.80 Hz and ^{4}J = 2.50 Hz, 2 x CH_{ortho}, benzyl phosphonate), 6.14 (d, 1H, ^{3}J = 7.90 Hz, H-1'), 4.91 (dd,

partially below solvent peak, 1H, ^3J = 7.55 Hz and ^3J = 4.75 Hz, H-2'), 4.62 (s, 1H, H-4'), 4.49 (dd, 1H, ^3J = 4.75 and ^3J = 1.60 Hz, H-3'), 4.09 – 4.04 (m, 4H, 2 x O-CH_2), 3.67 – 3.58 (m, 2H, ^3J= 5.35 Hz, N-CH_2 (propylamide), 3.24 (d, 2H, $^2J_{H,P}$ = 21.45 Hz, CH_2-P, benzyl phosphonate), 2.59 – 2.49 (m, 2H, $^3J_{X,A}$ = 5.35 Hz, O=C-$\underline{CH_2}$, propylamide), 1.29 (t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, D_2O) δ 174.9 (C=O), 174.4 (C=O), 157.9 (C-6), 155.3 (C-2), 150.7 (C-4), 143.9 (C-8), 138.8 (C_{para}, benzyl phosphonate), 133.1 (2 x CH_{ortho}, benzyl phosphonate), 129.6 (d, $^2J_{C,P}$ = 9.4 Hz, C_{ipso}, benzyl phosphonate), 123.1 (2 x CH_{meta}, benzyl phosphonate), 121.9 (C-5), 91.3 (C-1'), 87.4 (C-4'), 75.8 (C-2'), 74.7 (C-3'), 64.0 (2 x O-CH_2), 39.6 (N-CH_2, propylamide), 39.1 (O=C-$\underline{CH_2}$, propylamide), 34.1 (d, $^1J_{C,P}$ = 137.6 Hz, CH_2-P, benzyl phosphonate), 18.3 (2 x CH_3). ^{31}P-NMR (202 MHz, D_2O) δ 30.0. LC/ESI-MS: negative mode 576.3 ($[M-H]^-$), positive mode 578.2 ($[M+H]^+$).

22c: **4-[4-((*2S*,*3R*,*4S*,*5R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido)butylamido]benzylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: HBTU; yield over two steps: 360 mg, 61 %; 124 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.84 (br s, 1H, 4'-CONH), 9.05 (t, 2H, ^3J = 5.70 Hz, CONH, butylamide), 8.36 (s, 1H, H-2), 8.23 (s, 1H, H-8), 7.48 (d, 2H, ^3J = 8.55 Hz, 2 x CH_{ortho}, benzyl phosphonate), 7.38 (br s, 2H, 6-NH_2), 7.16 (dd, 2H, ^3J = 8.85 Hz and ^4J = 2.20 Hz, 2 x CH_{meta}, benzyl phosphonate), 5.95 (d, 1H, ^3J = 7.60 Hz, H-1'), 5.72 (br d, 1H, ^3J = 2.50 Hz, 2'-OH), 5.51 (br d, 1H, ^3J = 4.40 Hz, 3'-OH), 4.61 (dd, 1H, ^3J = 7.55 Hz and ^3J = 4.75 Hz, H-2'), 4.32 (d, 1H, ^3J = 1.60 Hz, H-4'), 4.14 (br s, 1H, H-3'), 3.95 – 3.89 (m, 4H, 2 x O-CH_2), 3.26 (dt, 2H, ^3J = 7.25 Hz and ^3J = 5.70 Hz, N-CH_2, butylamide), 3.12 (d, 2H, $^2J_{H,P}$ = 21.10 Hz, CH_2-P, benzyl phosphonate), 2.32 (t, 2H, ^3J = 7.25 Hz, O=C-$\underline{CH_2}$, butylamide), 1.79 (tt, 2H, ^3J = 7.25 Hz, CH_2, butylamide), 1.29 (t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.8 (C=O), 169.6 (C=O), 156.5 (C-6), 152.6 (C-2), 148.9 (C-4), 140.9 (C-8), 137.9 (C_{para}, benzyl phosphonate), 130.1 (2 x CH_{ortho}, benzyl phosphonate), 126.7 (d, $^2J_{C,P}$ = 9.4 Hz, C_{ipso}, benzyl phosphonate), 119.8 (2 x CH_{meta}, benzyl phosphonate), 119.1 (C-5), 88.0 (C-1'), 84.9 (C-4'), 73.4 (C-2'), 72.0 (C-3'), 61.5 (2 x O-CH_2), 38.5 (N-CH_2, butylamide), 33.8 (O=C-$\underline{CH_2}$, butylamide), 33.4 (d, $^1J_{C,P}$ = 137.6 Hz, CH_2-P, benzyl phosphonate), 25.3 (CH_2, butylamide), 16.4 und 16.3 (2 x CH_3). ^{31}P-NMR (202 MHz, DMSO-d_6) δ 27.1. LC/ESI-MS: negative mode 590.3 ($[M-H]^-$), positive mode 592.0 ($[M+H]^+$).

23a: **2-[(*2S*,*3R*,*4S*,*5R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido]ethylamidomethylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: HCTU; yield over two steps: 340 mg, 70 %; mp: 131°C. ^{1}H-NMR (500 MHz, MeOD) δ 8.38 (s, 1H, H-2), 8.25 (s, 1H, H-8), 6.11 (d, 1H, ^3J = 7.85 Hz, H-1'), 4.91 (dd, partly below solvent peak, 1H, ^3J = 7.85 Hz and ^3J = 4.70 Hz, H-2'), 4.62 (d, ^3J = 1.60 Hz, 1H, H-4'), 4.49 (dd, 1H, ^3J = 4.75 and ^3J = 1.25 Hz, H-3'), 4.17 – 4.13 (m, 4H, 2 x O-CH_2), 4.14 - 4.03 (AB-system with A d and B d, 2H, ^2J = 16.40 Hz, N-CH_2, ethylamide), 3.24 (AB-system with A dd and B dd, 2H, $^2J_{H,P}$ = 11.95 Hz and ^2J = 16.05 Hz, N-CH_2, methyl phosphonate), 1.32 (t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, MeOD) δ 173.3 (C=O), 171.4 (C=O), 157.8 (C-6), 154.1 (C-2), 150.5 (C-4), 142.9 (C-8), 121.4 (C-5), 90.7 (C-1'), 86.7 (C-4'), 75.4 (C-2'), 73.8 (C-3'), 64.5 (2 x O-CH_2), 43.3 (N-CH_2, ethylamide), 36.3 (d, $^1J_{C,P}$ = 157.1 Hz, CH_2-P, methyl phosphonate), 17.0 (2 x CH_3). ^{31}P-NMR (202 MHz, MeOD) δ 22.1. LC/ESI-MS: negative mode 486.4 ($[M-H]^-$), positive mode 488.3 ($[M+H]^+$).

23b: **3-[(*2S*,*3R*,*4S*,*5R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido]propylamidomethylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: PyBOP; yield over two steps: 150 mg, 30 %; mp: 118 °C. ^{1}H-NMR (500 MHz, MeOD) δ 8.37 (s, 1H, H-2), 8.34 (s, 1H, H-8), 6.06 (d, 1H, ^3J = 7.90 Hz, H-1'), 4.78 (dd, 1H, ^3J = 7.90 Hz and ^3J = 5.05 Hz, H-2'), 4.51 (d, ^3J = 1.60 Hz, 1H, H-4'), 4.38 (dd, 1H, ^3J = 5.00 Hz and ^3J = 1.25 Hz, H-3'), 4.15 – 4.07 (m, 4H, 2 x O-CH_2), 3.66 (AB-system with A dd and B dd, 2H, $^2J_{H,P}$ = 11.95 Hz and ^2J = 17.00 Hz, CH_2-P, methyl phosphonate), N-CH_2 (propylamide) not visible, covered by solvent, 2.54 (m, 2H, ^3J =

6.30 Hz, O=C-$\underline{CH_2}$, propylamide), 1.30 (2 x t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, MeOD) δ 173.7 (C=O), 172.8 (C=O), 157.9 (C-6), 154.5 (C-2), 150.5 (C-4), 142.9 (C-8), 121.5 (C-5), 90.7 (C-1'), 86.8 (C-4'), 75.3 (C-2'), 73.7 (C-3'), 64.4 and 64.3 (2 x O-CH_2), 37.1 (N-CH_2, propylamide), 36.6 (O=C-$\underline{CH_2}$, propylamide), 35.6 (d, $^1J_{C,P}$ = 157.1 Hz, N-CH_2-P, methyl phosphonate), 17.0 and 16.9 (2 x CH_3). ^{31}P-NMR (202 MHz, MeOD) δ 22.5. LC/ESI-MS: negative mode 500.3 ($[M-H]^-$), positive mode 502.4 ($[M+H]^+$).

23c: **4-[(*2S*,*3R*,*4S*,*5R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofurane-2-carboxamido]butylamidomethylphosphonic acid diethyl ester**. Coupling reagent used in the synthesis: PyBOP; yield over two steps: 380 mg, 65 %; mp: 128 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.02 (t, 1H, ^3J = 5.70 Hz, 5'-CONH), 8.36 (s, 1H, H-2), 8.21 (s, 1H, H-8), 8.18 (t, 1H, ^3J = 6.00 Hz, CONH), 7.38 (br s, 2H, 6-NH_2), 5.95 (d, 1H, ^3J = 7.55 Hz, H-1'), 5.72 (d, 1H, ^3J = 4.10 Hz, 2'-OH), 5.50 (d, 1H, ^3J = 6.30 Hz, 3'-OH), 4.91 (dd, 1H, ^3J = 6.95 Hz and ^3J = 4.70 Hz, H-2'), 4.31 (d, ^3J = 0.95 Hz, 1H, H-4'), 4.12 (pseudo-t, 1H, ^3J = 7.25 and ^3J = 3.75 Hz, H-3'), 4.02 – 3.96 (2 x q, 4H, 2 x O-CH_2), 3.54 (pseudo-q, 2H, $^2J_{H,P}$ = 11.35 Hz and ^2J = 17.30 Hz and ^3J = 6.00 Hz, N-CH_2, methyl phosphonate), 3.19 (dt, 2H, ^3J = 6.90 Hz and ^3J = 5.35 Hz, N-CH_2, butylamide), 2.15 (t, 2H, ^3J = 7.25 Hz, O=C-$\underline{CH_2}$, butylamide), 1.70 (tt, 2H, ^3J = 6.95 Hz and 7.55 Hz, CH_2, butylamide), 1.32 (t, 6H, 2 x CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 171.9 (C=O), 169.6 (C=O), 156.5 (C-6), 152.7 (C-2), 148.9 (C-4), 140.9 (C-8), 119.8 (C-5), 88.1 (C-1'), 84.9 (C-4'), 73.4 (C-2'), 72.1 (C-3'), 61.8 (2 x O-CH_2), 38.2 (N-CH_2, butylamide), 34.0 (d, $^1J_{C,P}$ = 154.2 Hz, CH_2-P, methyl phosphonate), 32.6 (O=C-$\underline{CH_2}$, butylamide), 25.6 (CH_2, butylamide), 16.4 and 16.3 (2 x CH_3). ^{31}P-NMR (202 MHz, DMSO-d_6) δ 23.6. LC/ESI-MS: negative mode 526.3 ($[M-H]^-$), positive mode 514.4 ($[M+H]^+$).

Experimental procedures for testing of compounds at $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors

Cell culture

Astrocytoma cell lines stably transfected with either the human $P2Y_2$, the human $P2Y_4$, or the rat $P2Y_6$ receptor were cultured in Dulbecco's modified Eagle medium (DMEM, Cambrex BE-12 604F) containing 800 µg/mL gentamicin (G 418, Calbiochem), 10 % fetal bovine serum (Sigma Aldrich) and 2 mM ultraglutamine (Cambrex) at 37 °C in 5 % CO_2. Astrocytoma 1321N1 cell lines stably expressing the human $P2Y_4$-receptor, or the rat $P2Y_6$ receptor, respectively, were obtained from G.A. Weisman, University of Missouri-Columbia, USA.[3] 1321 N1 cells stably expressing the human $P2Y_2$ receptor were made in our laboratory by Petra Hillmann.[4]

Calcium mobilization

Test compounds were investigated in functional assays by measuring inhibition of $P2Y_2$, $P2Y_4$ or $P2Y_6$ receptor-mediated intracellular calcium mobilization by test compounds using a FLUOstar® plate reader, or a NOVOstar® plate reader, respectively, both equipped with one injector (BMG LabTechnologies, Offenburg, Germany) essentially as previously described.[5,6] When cells were 80 % confluent, they were rinsed off the culture flasks with cell culture medium. They were then left in the incubator (37 °C, 5 % CO_2) for 30 to 60 min before they were centrifuged (200 x g, 5 min, 4 °C). The cell pellet was resuspended in 994 µL Krebs-HEPES buffer at 37 °C. Krebs-HEPES buffer consisted of NaCl (118.6 mM), KCl (4.7 mM), KH_2PO_4 (1.2 mM), $NaHCO_3$ (4.2 mM), D-glucose (11.7 mM), Hepes (free acid, 10 mM), $CaCl_2$ (1.3 mM) and $MgSO_4$ (1.2 mM). A mixture of 3 µL of a 1 mM solution of Fura-2 AM (Molecular Probes, Eugene, OR) and 3 µL of a 20 % solution of Pluronic F-127 (Molecular Probes) in DMSO was added to obtain a final volume of 1 mL. Cells were shaken for 1 h at rt (600 rpm, under exclusion of light), subsequently centrifuged (3500 rpm, few seconds, 4 °C), washed twice with Krebs-HEPES buffer, resuspended in 1 mL Krebs-HEPES buffer, diluted and plated into black 96-well plates (Nunc Maxisorp) with a density of 17,000 cells/well. Microplates were kept at rt. Antagonistic activity

of the test compounds was determined as follows. Cells (160 µL per well) were preincubated with three concentrations of the test compound (1, 10 and 100 µM, 20 µL per well, each in triplicate) for at least 20 min before injection of the physiological ligand (1 µM UTP for the $P2Y_2$, 3 µM UTP for the $P2Y_4$, 3 µM UDP for the $P2Y_6$ receptor, 20 µL per well). The final volume was 200 µL per well. Fluorescence was measured at 520 nm (bandwidth 20 nm) after excitation at 320 nm for 56 intervals of 0.4 seconds each. For compounds which showed an inhibition of more than 40 % at a concentration of 10 µM full curves were determined with seven or eight different concentrations spanning three orders of magnitude to determine IC_{50} values. Receptor agonists will also show an inhibitory activity in this assay due to receptor desensitization.

Three separate experiments were performed, each in triplicate. The final DMSO concentration in the assays never exceeded 2.5 %. This concentration was found out to have no effect on the cells and the fluorescence signals in antagonist assays.

Data analysis

IC_{50} values for antagonists were calculated by nonlinear regression using Prism® 3.0 (GraphPad Software, San Diego, CA, USA).

Table 2. Inhibition of agonist-induced calcium mobilization of nucleotide mimetics at $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors

Compound	**hP2Y$_2$** % inhibition ± SEM (n=3) of UTP-induced[a] calcium mobilization at 100 μM (EC_{50} of UTP 0.861 μM)	**hP2Y$_4$** % inhibition ± SEM (n=3) of UTP-induced[b] calcium mobilization at 100 μM (EC_{50} of UTP 1.36 μM)	**rP2Y$_6$** % inhibition ± SEM (n=3) of UDP-induced[b] calcium mobilization at 100 μM (EC_{50} of UDP 0.730 μM)
19a	**22** ± **6**	**4** ± 3	**- 10** ± 10
19b	**10** ± 10	**14** ± 6	**7** ± 7
19c	**6** ± 3	**7** ± 1	**- 8** ± 11
20a	c	**38** ± 10	**17** ± 16
20b	**43** ± 2	**6** ± 3	**- 3** ± 2
20c	c	**21** ± 10	**- 3** ± 11
21a	**-7** ± 7	**8** ± 12	**9** ± 13
22a	c	**7** ± 1	**6** ± 6
22b	**46** ± 7	**- 18** ± 27[d]	**- 15** ± 0
22c	**37** ± 6	**- 14** ± 25[d]	**- 13** ± 8
23a	**61** ± 8	n.d.	n.d.
23b	**29** ± 2	n.d.	n.d.
23c	**32** ± 2	n.d.	n.d.
Suramin	IC_{50} ca. 50 μM[3]	inactive (up to 100 μM)[7]	IC_{50} 27 μM (human)[8]
Reactive Blue 2	IC_{50} 12.0 ± 5.7 μM[4]	IC_{50} 5.75 ± 0.69 μM	IC_{50} 4.34 ± 0.89 μM
PPADS	inactive (up to 300 μM)	IC_{50} 73 μM	IC_{50} 69 μM (human)

[a] 1 μM UTP
[b] 3 μM UTP ($P2Y_4$) or UDP ($P2Y_6$)
[c] no concentration-dependent inhibition (0.1-100 μM).
[d] n=2

Table 3. Effects of test compounds on intracellular calcium levels in $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptor-expressing astrocytoma 1321N1 cells

Compound	human $P2Y_2$ % activation ± SEM (n=2) at 100 µM in relation to the maximal UTP effect	human $P2Y_4$ % activation ± SEM (n=2) at 100 µM in relation to the maximal UTP effect	rat $P2Y_6$ % activation ± SEM (n=2) at 100 µM in relation to the maximal UDP effect
19a[a]	**- 21** ± 19	**- 42** ± 34	**- 7** ± 1
19b	**14** ± 6	**- 6** ± 15	**- 5** ± 1
20a	**58** ± 22	**46** ± 8	**12** ± 2
20b	**13** ± 11	**49** ± 2	**- 16** ± 0
22b	**16** ± 9	**11** ± 4	**- 7** ± 1
23a	**33** ± 9	**- 2** ± 8	**6** ± 4

[a]Negative values may be due to nucleotidase-inhibitory activity. We have previously observed this effect with other NTPDase inhibitors.

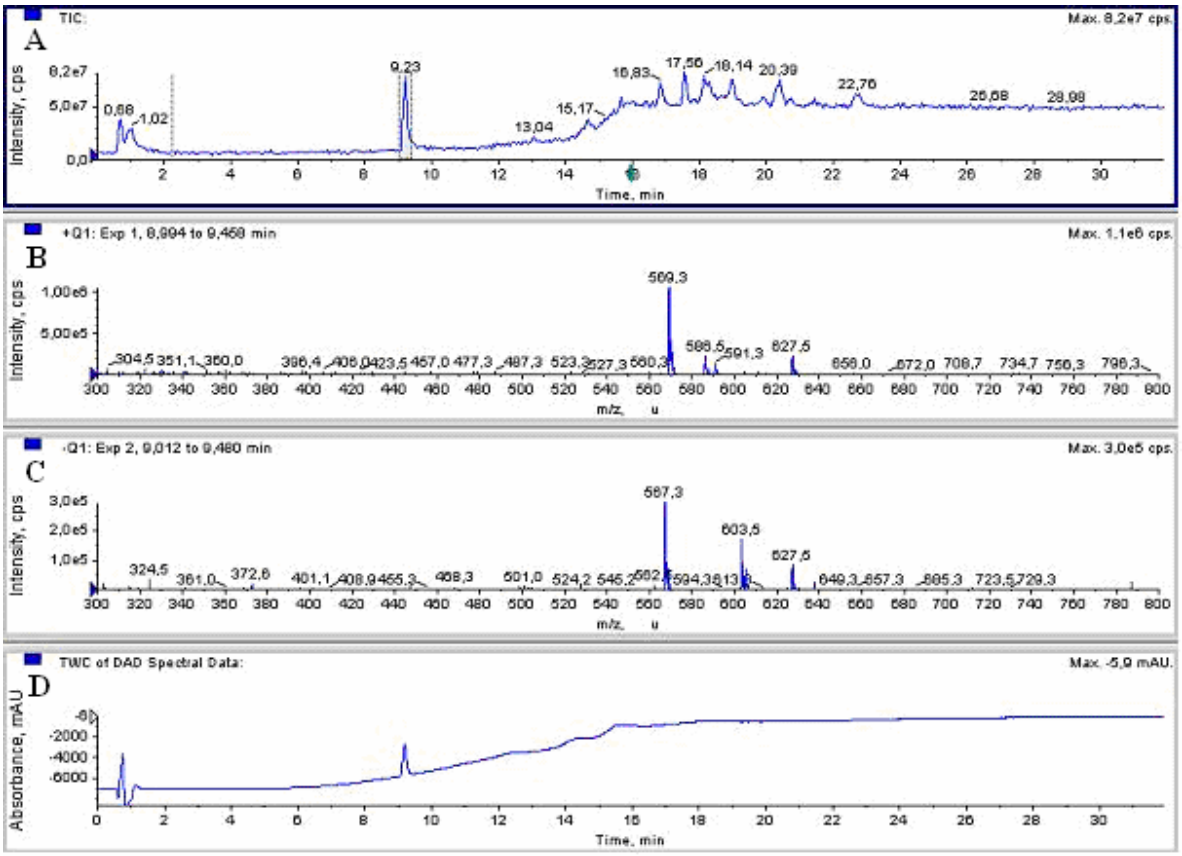

Figure 1. Representative HPLC chromatogram and corresponding MS spectra of compound **19c** after incubation for 24 h in simulated artificial gastric acid.

(**A**) Total ion count (TIC). HPLC chromatogram, detection by MS. The peak at the retention time of 9.23 min belongs to compound **19c** (M= 568 g/mol).

(**B**) Corresponding MS spectrum of the peak at the retention time of 9.23 min in positive mode $[M+H]^+$ (m/z=569 u) of compound **19c**.

(**C**) Corresponding MS spectrum of the peak at the retention time of 9.23 min in negative mode $[M-H]^-$ (m/z=567 u) of compound **19c**.

(**D**) Total wavelength count (TWC). HPLC chromatogram, detection by DAD.

No degradation products were detectable. The peak at the retention time of 1.02 min belongs to pepsin from the artificial gastric acid.

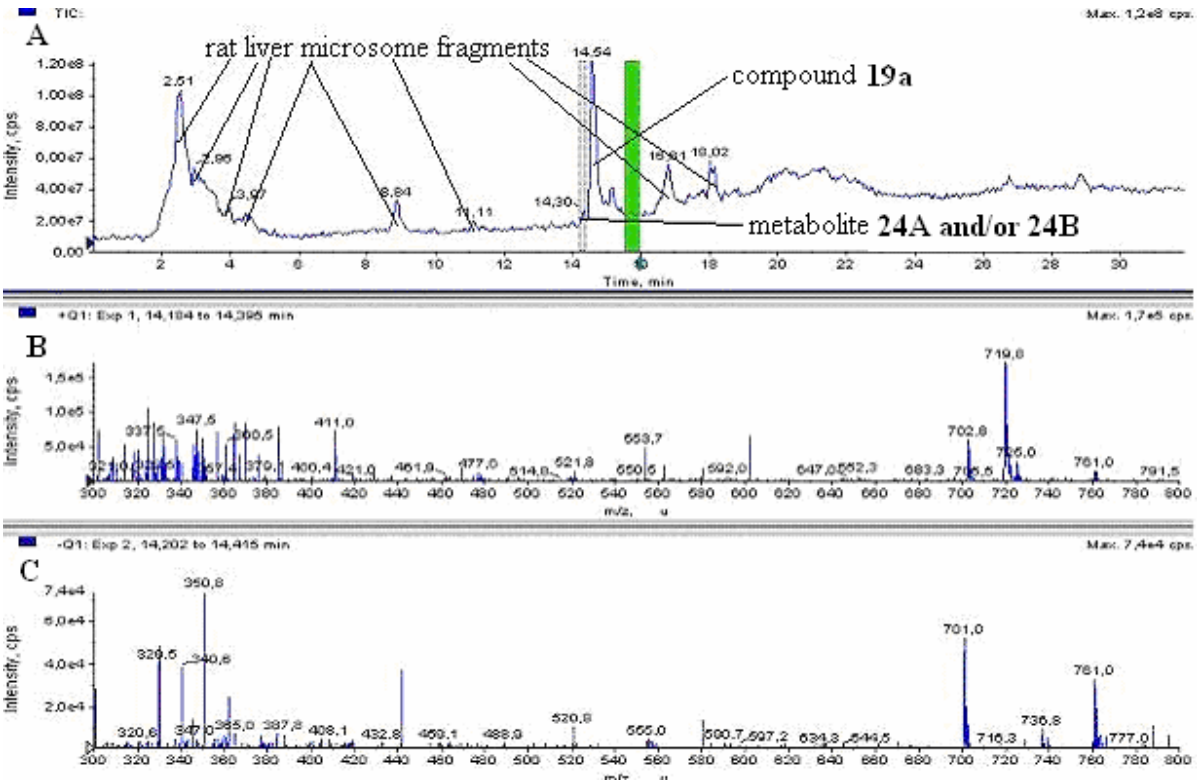

Figure 2. Representative HPLC chromatogram and corresponding MS spectra of compound **19a** after 24 h incubation with rat liver microsomes at 37° C.

(**A**) Total ion count (TIC). HPLC chromatogram, detection by MS. The peak at the retention time of 14.54 min belongs to compound **19a** (M= 540 g/mol) and the peak at the retention time of 14.30 min belongs to metabolite **24A** and/or **24B** (m/z=702 u).

(**B**) Corresponding MS spectrum of the peak at the retention time of 14.30 min in positive mode $[M+H]^+$ (m/z=703 u) of metabolite **24A** and/or **24B**.

(**C**) Corresponding MS spectrum of the peak at the retention time of 14.30 min in negative mode $[M-H]^-$ (m/z=701 u) of metabolite **24A** and/or **24B**.

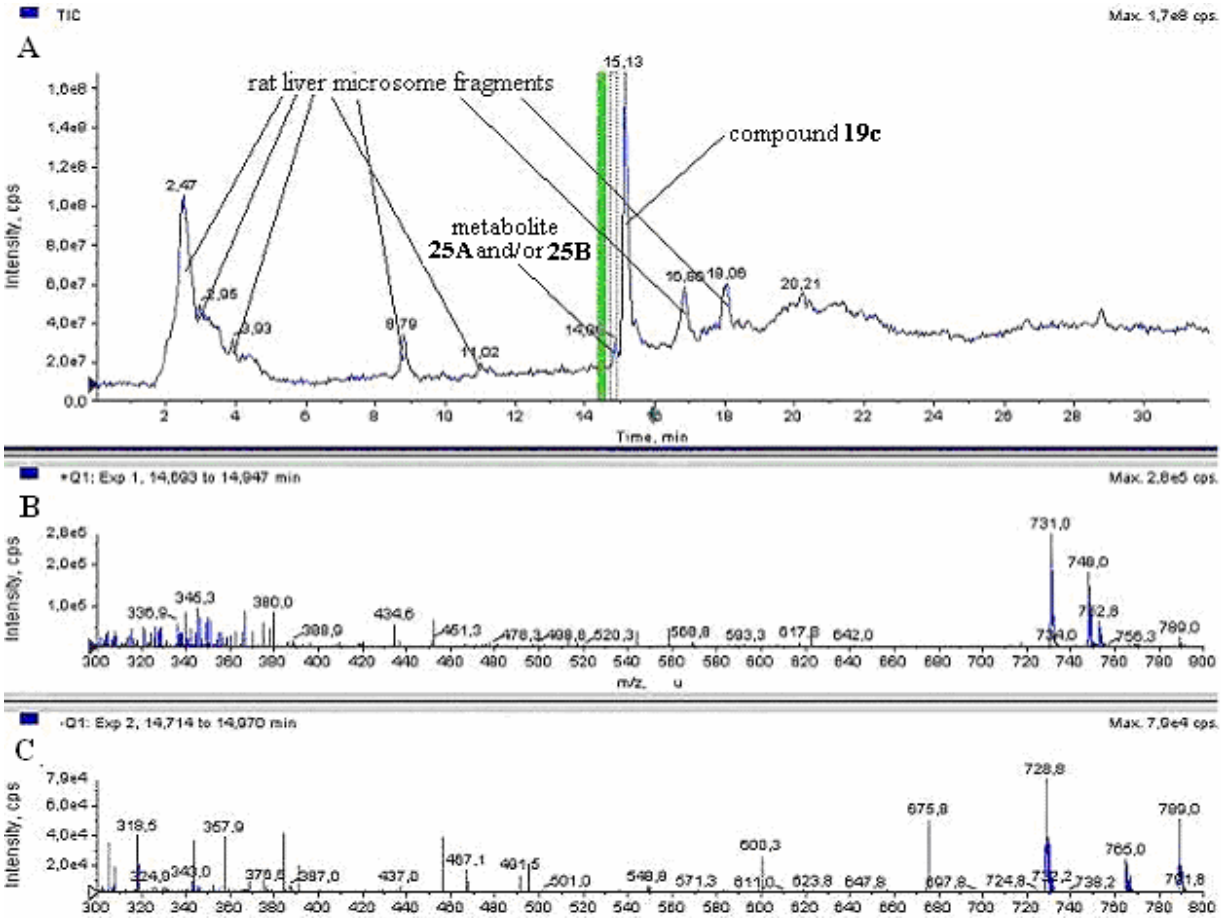

Figure 3. Representative HPLC chromatogram and MS spectra of compound **19c** after 24 h incubation with rat liver microsomes at 37° C.

(**A**) Total ion count (TIC). HPLC chromatogram, detection by MS. The peak at the retention time of 15.13 min belongs to compound **19c** (M= 568 g/mol) and the peak at the retention time of 14.80 min belongs to metabolite **25A** and/or **25B** (m/z=730 u).

(**B**) Corresponding MS spectrum of the peak at the retention time of 14.80 min in positive mode $[M+H]^+$ (m/z=731 u) of metabolite **25A** and/or **25B**.

(**C**) Corresponding MS spectrum of the peak at the retention time of 14.80 min in negative mode $[M-H]^-$ (m/z=729 u) of metabolite **25A** and/or **25B**.

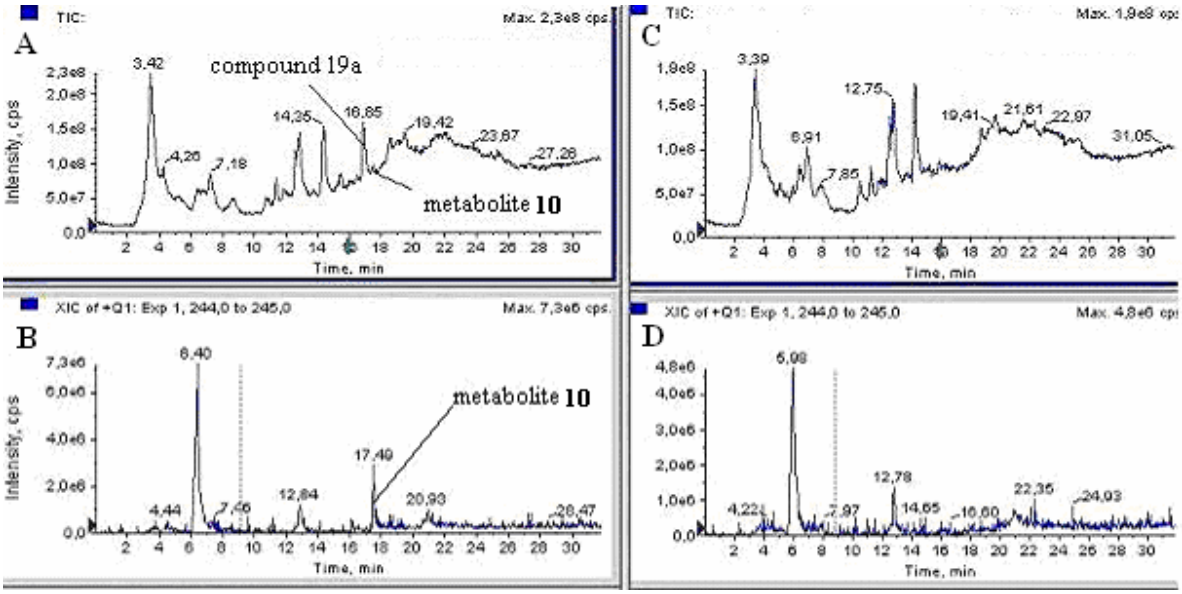

Figure 4. Detection of metabolite **10** in samples with and without compound **19a** incubated 24 h with rat liver microsomes at 37° C.

(A) Total ion count (TIC). HPLC chromatogram of the sample with compound **19a** incubated 24 h with rat liver microsomes at 37° C, detection by MS. The peak at the retention time of 16.85 min belongs to compound **19a** (M= 540 g/mol) and the peak at the retention time of 17.49 min belongs to metabolite **10** (M=244 g/mol).

(B) HPLC-MS chromatogram. Scan on metabolite **10** (m/z=244 u) in positive mode. At the retention time of 17.49 min metabolite **10** is detectable.

(C) Total ion count (TIC). HPLC chromatogram of the sample without compound **19a** incubated 24 h with rat liver microsomes at 37° C, detection by MS. Metabolite **10** is not detectable.

(D) HPLC-MS chromatogram. Scan on metabolite **10** in positive mode. Metabolite **10** is not detectable.

Metabolite **10** (M= 244 g/mol) was only detectable in the samples with compound **19a** (and **19c**) incubated with rat liver microsomes. It was not detectable in the blank values without compounds **19a** (and **19c**) and in the blank values without liver microsomes.

References

(1) Marwood, R. D.; Riley, A.M.; Jenkins, D.J.; Potter, B.V.L. Synthesis of adenophostin A and congeners modified at glucose. *J. Chem. Soc., Perkin Trans 1* **2000**, 1935-1947.

(2) Smith, M.; Rammler, D.H.; Goldberg, I.H.; Khorana, H.G. Polynucleotides. XIV. Specific synthesis of the C3'-C5' internucleotide linkage. Synthesis of uridylyl(3' -> 5')-uridine and uridylyl-(3' -> 5')-adenosine. *J. Am. Chem. Soc.* **1962**, *84*, 430-440.

(3) Charlton, S. J.; Brown, C.A.; Weisman, G.A.; Turner, J.T.; Erb, L.; Boarder, M.R. Cloned and transfected P2Y4 receptors: characterization of a suramin and PPADS-insensitive response to UTP. *Br. J. Pharmacol.* **1996**, *119* , 1301-1303.

(4) Hillmann, P. Molekulare Basis der Aktivierung und Modulation des $P2Y_2$-Nukleotid-Rezeptors (Molecular basis of activation and modulation of the $P2Y_2$ nucleotide receptor). Ph.D. thesis, University of Bonn, **2007**.

(5) Kassack, M. U.; Höfgen, B.; Lehmann, J.; Eckstein, N.; Quillan, J.M.; Sadee, W. Functional screening of G protein-coupled receptors by measuring intracellular calcium with a fluorescence microplate reader. *J. Biomol. Screen.* **2002**, *7*, 233-246.

(6) Kaulich, M.; Streicher, F.; Mayer, R.; Müller, I.; Müller, C.E. Flavonoids - novel lead compounds for the development of $P2Y_2$ receptor antagonists. *Drug Dev. Res.* **2003**, *59*, 72-81.
(7) Communi, D.; Motte, S.; Boeynaems, J.M.; Pirotton, S. Pharmacological characterization of the human $P2Y_4$ receptor. *Eur. J. Pharmacol.* **1996**, *317*, 383-389.
(8) Robaye, B.; Boeynaems, J.M.; Communi, D. Slow desensitization of the human $P2Y_6$ receptor. *Eur. J. Pharmacol.* **1997**, *329*, 231-236.