51. Nitrostyrene Derivatives of Adenosine 5′-Glutarates as Selective Inhibitors of the Epidermal Growth Factor Receptor Protein Tyrosine Kinase

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The syntheses and biological activities of some nitrostyrene derivatives of adenosine 5′-glutarates, a novel class of selective, bi-substrate-type inhibitors of the EGF receptor protein tyrosine kinase, are described. The most interesting compounds (**14–16**) were able to inhibit the EGF-R tyrosine kinase with IC_{50} values around 1 μM. Only marginal inhibition of the tyrosine kinases *v-abl* and *c-src* and of the serine/threonine kinase **PK**C was observed. Compounds **8**, **9**, **11**, and **12** – lacking the adenosine moiety – were ten times less active than the most potent derivatives, whereas **17** – lacking the nitrostyryl part – showed no inhibitory activity at all. Most of the compounds showed potent antiproliferative activity against an EGF-dependent mouse keratinocyte cell line.

Introduction. – Cell proliferation is tightly regulated by growth factors and hormones constituting growth signals. Growth signals are transmitted across the cell membrane to the nucleus *via* signal transduction pathways. This applies to the proliferation of normal as well as malignant cells, whose growth is out of control. In recent years, broad knowledge about these signalling events converting a normal cell to a malignant cell was gained. In the response of cells to such regulatory signals, phosphorylation of proteins on tyrosine residues by protein tyrosine kinases (PTK) is of prime importance.

Among the best understood members of the protein tyrosine kinase families are the growth factor receptor PTK's [1–6] such as the epidermal growth factor receptor (EGF-R), platelet-derived growth factor receptor (PDGF-R), and insulin receptor (I-R) PTK's. Normal epithelial cells require epidermal growth factor (EGF) as a signal for cell division. The binding of the EGF to the extracellular domain of the EGF receptor activates its intracellular tyrosine kinase domain [1] [6]. This interaction triggers a series of regulatory events that are essential for the regulation of cellular proliferation. Abnormally expressed or deregulated EGF-R PTK is thought to play an important role in the loss of growth control associated with tumors of epithelial origin (*e.g.* breast tumors) [1]. Therefore, the EGF-R PTK was selected as target for the rational design of PTK inhibitors, which could possibly be used as anti-tumor therapeutics.

Concept and Design of Inhibitors. – Protein tyrosine kinases catalyze the direct transfer of the γ-phosphate group from ATP to a tyrosine moiety in a substrate peptide molecule. For this transfer, a transition state is postulated with a pentacoordinated γ-P-atom and with the β- and γ-phosphate groups forming a complex with bivalent metal ions, usually Mg^{2+} or Mn^{2+} (*Fig.*) [7–9].

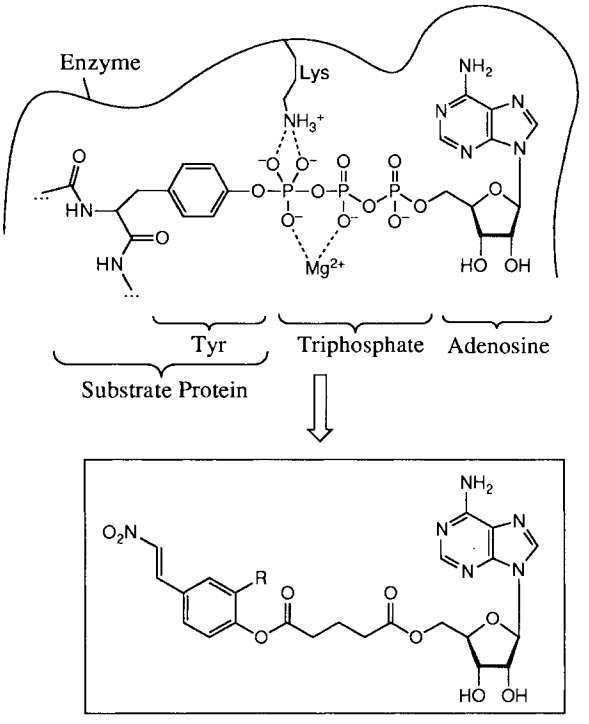


Figure. *Postulated transition state during the phosphate transfer by PTK* (adapted from [12]), *and schematic representation of possible bi-substrate inhibitors*

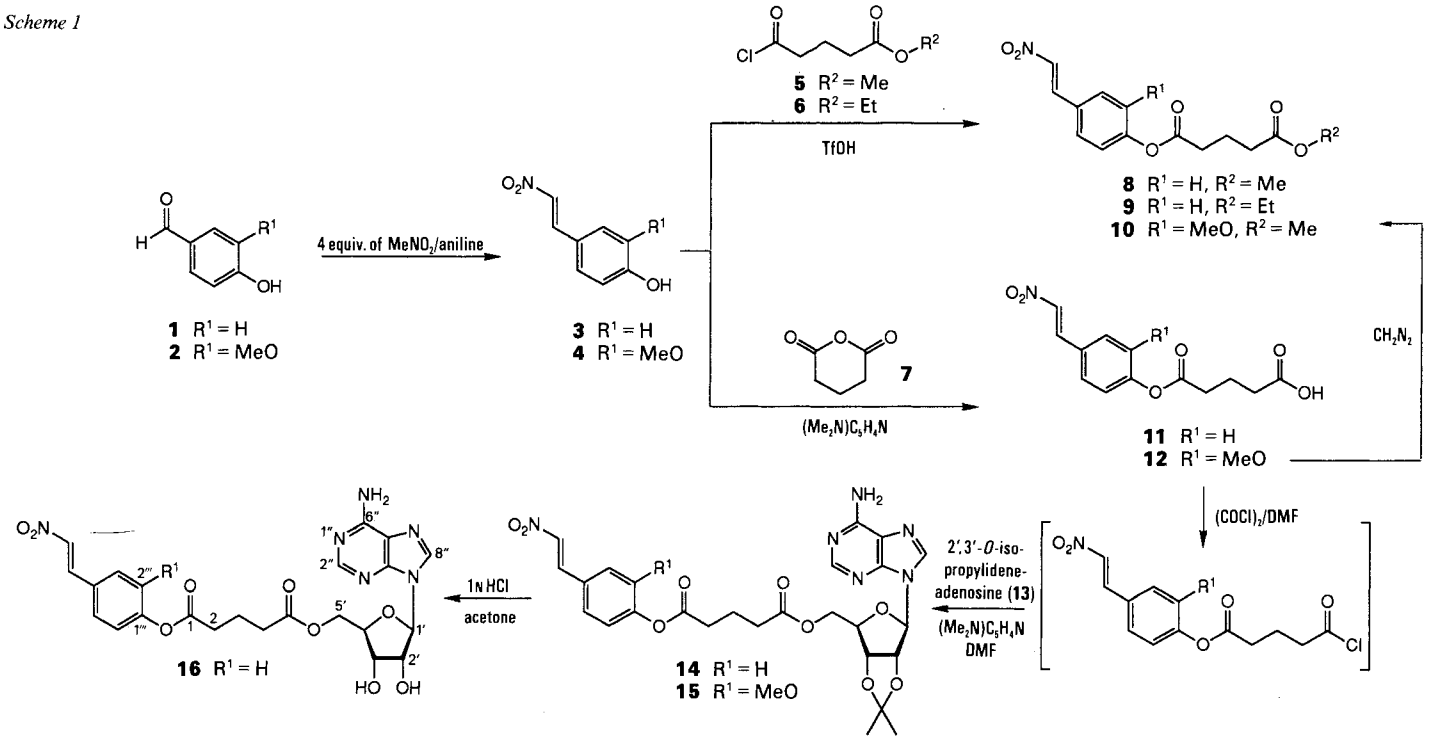
We approached the rational design of EGF-R inhibitors by attempting the synthesis of multi-substrate complex analogues consisting of an inhibitory tyrosine mimic or analogue combined with a triphosphate mimic or spacer. Such bi-substrate-type inhibitors containing structural elements of both ATP and tyrosine have the potential of mimicking the postulated transition state and were already shown to lead to compounds with moderate to potent inhibitory properties against tyrosine kinases [10–12].

Nitrostyrenes were recently identified as moderately potent inhibitors of EGF-R PTK. Their combination with a sulfonylbenzoyl moiety as a triphosphate mimic/spacer led to multi-substrate complex-type inhibitors which were highly potent against the EGF-R PTK. In addition, these inhibitors were highly selective with respect to other tyrosine kinases (*v-abl*, *c-src*) and serine/threonine kinases, such as protein kinase C (PKC) or cyclic AMP dependent protein kinase (PKA) [12].

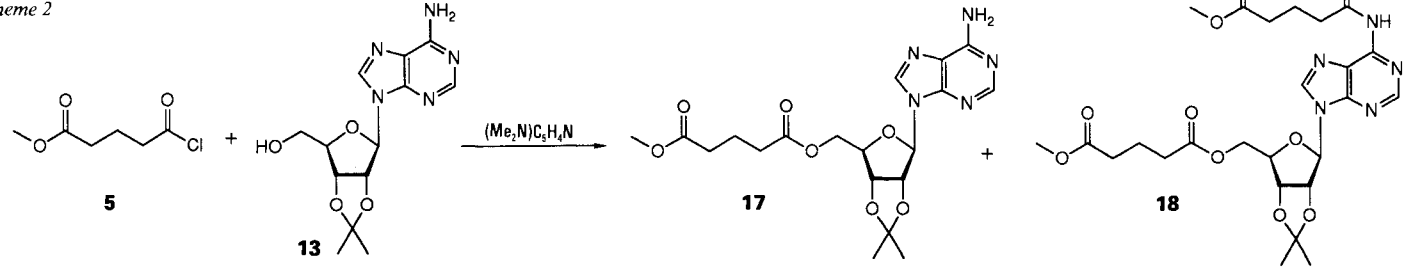
In the present study, we used the nitrostyrenes **3** and **4** as tyrosine mimics, while glutaric acid was used as the substitute for the triphosphate unit. This led to two series of multi-substrate complex-type inhibitors of the EGF-R PTK.

Syntheses. – Starting from 4-hydroxybenzaldehyde (**1**), the nitrostyrene **3** was synthesized in 69% yield in a nitro-aldol condensation with aniline as catalyst according to a literature procedure [13] (*Scheme 1*). Similarly, **4** was obtained from **2**. Following a procedure by *Effenberger* and *Klenk* [14], **3** was then esterified with O^5-methyl glutar-1-yl

Scheme 1



Scheme 2

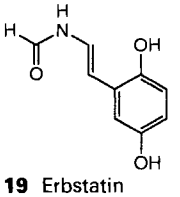


cloride (**5**) or O^5-ethyl glutar-1-yl cloride (**6**) in the presence of trifluoromethanesulfonic acid (TfOH) as acylation catalyst to give **8** and **9**, respectively. The base-sensitive phenyl-ester linkage was partly cleaved during workup with aqueous NaOH solution. To connect adenosine to the nitrostyrene-glutaryl unit, we needed the free acid **11** or its corresponding chloride. Selective cleavage of the aliphatic ester linkage in **8** or **9** was unsuccessful due to the lability of the phenyl ester. Crude **11** could, however, be obtained in 91% yield directly from **3** by reaction with an equimolar amount of glutaric anhydride (**7**) and 4-(dimethylamino)pyridine ($(Me_2N)C_5H_4N$) as acylation catalyst. The crude product contained 14% of starting material which could not be removed by chromatography, because **11** decomposed on silica gel. Recrystallization from boiling MeOH, however, gave the free acid **11** as yellow crystals in 94% purity. Acid **12** was obtained in a similar way from **4** and was esterified to **10**.

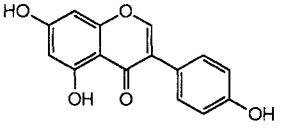
Compound **11** was then converted to the corresponding acyl chloride by the mild oxalyl chloride method [11]. This procedure uses an excess of $(COCl)_2$ with DMF as catalyst in an apolar aprotic solvent between 0° and r.t. The resulting acyl chloride was added directly to an equimolar amount of 2′,3′-*O*-isopropylideneadenosine (**13**) in DMF containing $(Me_2N)C_5H_4N$ as catalyst [11] to give crude **14**. Column chromatography gave the pure protected adenosine ester **14** in 10% overall yield. Adenosine ester **15** was synthesized analogously from **12**. The 2′,3′-*O*-isopropylidene group of **14** could not be removed with BCl_3 [15]; the phenyl-ester bond was cleaved instead. Deprotection of **14** was achieved by treatment with 1N HCl in acetone[1]), followed by neutralization with a phosphate-buffer, freeze-drying, and column chromatography to give **16.**

For the comparison of the biological activity of a combination of the glutaryl and the adenosine moieties with compounds **14** and **15**, 2′,3′-*O*-isopropylideneadenosine 5′-(methyl glutarate) (**17**) was synthesized in low yield from O^5-methyl glutar-1-yl chloride (**5**) and 2′,3′-*O*-isopropylideneadenosine (**13**) with $(Me_2N)C_5H_4N$ as catalyst. As a by-product, the diglutaryl product **18** was also formed (*Scheme 2*).

Results and Discussion. – *Enzymatic Activity.* The nitrostyryl-glutarate derivatives were tested for inhibition of the EGF-R tyrosine kinase as previously described using A431 membranes as the enzyme source and the octapeptide angiotensin II as the phosphoryl acceptor substrate [16] [12] [17]. Compounds were further tested within the PTK family using purified recombinant *v-abl* kinase and [Val^5]angiotensin II [12] [17] and recombinant *c-src* and the random polymer Glu,Tyr (4:1) as substrates [18]. For the determination of selectivity towards the serine/threonine protein kinase C (PKC), histone II-S was used as previously described [19].



19 Erbstatin



20 Genistein

[1]) Even under these conditions, substantial ester cleavage was observed.

Table. *Inhibitory and Antiproliferative Activities of Compounds* **3**–**17**, **19**, *and* **20**

	Inhibition of protein kinases, IC_{50} [μM]				Antiproliferative activity against MK cells, IC_{50} [μM]
	EGF-R	*v-abl*	*c-src*	PKC	
3	11.5	> 100	75	> 500	6.5
4	7.9	> 100	75	> 500	5.9
8	5.3	> 50	> 100	> 500	14.4
9	5.8	> 100	> 100	> 500	9.6
10	1.2	> 50	50	220	23.6
11	11.2	> 100	≈ 100	390	5.4
12	4.7	> 100	> 50	> 500	5.8
14	0.66	> 100	> 100	95	15.6
15	0.6	> 100	> 100	78	4.4
16	1.5	50	24	43	22.2
17	> 50	> 100	> 100	> 500	> 50
Erbstatin (**19**)	12.7	> 100	> 100	> 100	13.6
Genistein (**20**)	1.0	10	75	15	9.1

As shown in the *Table*, nitrostyrenes **3** and **4** were found to be moderately potent and selective inhibitors of the EGF-R PTK. Their IC_{50} values were comparable to that of the natural inhibitor erbstatin (**19**) [20]. When tested for selectivity, only marginal inhibition of the *c-src* PTK, but no inhibition of either the *v-abl* PTK or the serine kinase PKC was found. In general, no significant increase of EGF-R kinase inhibition was observed when the glutaryl moiety was added to the nitrostyrenes **3** or **4** with the exception of the methyl ester **10**. Thus, the nitrostyryl glutarates **8**, **9**, **11**, and **12** showed IC_{50} values comparable to their parent compounds **3** and **4**, respectively. Introduction of a MeO substituent at C(2) of the nitrostyryl part (compounds **10** and **12**) improved the enzymatic activity by a factor of 2 to 4.

A more than 10-fold increase of EGF-R kinase inhibition was observed when the 2′,3′-*O*-isopropylideneadenosine moiety was connected to the free acid **11**. Compound **14** had an IC_{50} value of 0.66 μM compared to 11.2 μM of **11** or 11.5 μM of the parent nitrostyrene **3**. The corresponding MeO-substituted analogue **15** had approximately the same IC_{50} value as **14**. Removal of the isopropylidene group in **14** did not significantly alter the enzymatic activity: **16** had an IC_{50} value of 1.5 μM. All these values are comparable to that of the natural inhibitor genistein (**20**), which was used as a reference compound. Compounds **14**–**16** seem to act as bidentate inhibitors, as they are also much more active than the glutarylated adenosine derivative **17**, which showed no inhibitory activity.

Interestingly, the adenosine derivatives **14**–**16** showed decreased selectivity toward the *v-abl* and *c-src* tyrosine kinases and especially toward the serine/threonine kinase PKC. This is not surprising since it is well known that there is high homology within the ATP binding sites of different protein kinases. We, therefore, suggest that in PKC, these adenosine derivatives might interact primarily with the ATP binding sites, whereas in the EGF-R PTK, binding to both, substrate and ATP binding sites can be assumed. Preliminary data of enzyme kinetics support these assumptions: **14** was competitive with ATP, whereas angiotensin kinetics showed a mixed type of inhibition (data not shown).

Antiproliferative Activity. The nitrostyryl glutarates were tested for inhibition of tumor-cell proliferation using mouse epidermal keratinocytes (BALB/MK cells). These

cells strongly depend on EGF for their proliferation [21]. In general, nitrostyryl glutarates with inhibitory activities against the EGF-R tyrosine kinase also showed antiproliferative activities against the MK cells. Inhibition was observed at IC_{50} values between 4.4 and 23.6 μM (see *Table*). As expected, compound **17**, which was inactive at the enzyme level, was devoid of any antiproliferative effect. However, no structure-activity relationship can be drawn from the cellular data. Whether the antiproliferative effects of this class of compounds is fully due to EGF-R kinase inhibition, or whether effects unrelated to kinase inhibition are responsible for cell proliferation, has to be clarified by further experiments (*e.g.* inhibition of EGF-induced EGF-R autophosphorylation in intact cells).

Conclusion. – The results presented here demonstrate that the glutarate moiety can be considered as a suitable triphosphate mimic/spacer, and that the nitrostyryl part is a good substitute for the tyrosine-containing substrate of the EGF-R kinase. Compounds **14–16** indeed seem to act as multi-substrate complex analogues.

The technical assistance of *B. Adam*, *J. Bohn*, *N. Martin*, and *R. Reuter*, *Ciba-Geigy AG*, and the help of Dr. *Th. Meyer* and Dr. *U. Regenass*, *Ciba-Geigy AG*, are gratefully acknowledged. This work was supported by the *Swiss National Science Foundation* (grant No. 20.29817.90).

Experimental Part

General. All chemicals were purchased from *Fluka AG* or *Merck GmbH* in *purum* or *puriss. p.a.* quality. Solvents used in reactions were distilled and dried. Solvents used for column chromatography (industrial grade) were distilled once. $CHCl_3$ and CH_2Cl_2 were passed through a 15-g column filled with glass wool, neutral aluminium oxide, molecular sieve (3 Å and 4 Å, activated in microwave oven) and sand in order to remove H_2O and stabilizer (EtOH). Org. extracts were dried (Na_2SO_4) and evaporated ($\leq 30°/\geq 15$ mbar) and the residues dried under high vacuum (≥ 0.1 mbar). TLC: *Merck* precoated glass plates, 0.25 mm silica gel *60* F_{254}, detection in UV after exposure to I_2 vapors. Column chromatography: flash columns with H_2O cooling; overpressure *ca.* 0.3 bar; *Merck* silica gel *60*, 40–63 μm, or *Chemische Fabrik Uetikon* silica gel *C560*, 35–70 μm. M.p.: *Kofler* hot stage with polarizing filters; uncorrected. UV Spectra: 1-cm or 1-mm quartz cuvettes; *Perkin-Elmer Lambda 9*. IR Spectra: KBr pellets; *Perkin-Elmer 1310*. NMR Spectra: *Varian VXR-400* (^{1}H: 400 MHz; ^{13}C: 101 MHz), *Varian Gemini-300* (^{1}H: 300 MHz; ^{13}C: 75 MHz); δ in ppm rel. to internal TMS (0 ppm); digital resolution for coupling constants, 0.2 Hz/point; multiplicities of ^{13}C resonances from APT experiments; *means that assignments may be interchanged. MS: *VG-70-250*. HR-FAB-MS: *VG-70-4SE*; matrix, 3-nitrobenzyl alcohol (NBA); internal standard, polypropylene glycol (*PPG-425*); average of 2 independent measurements.

Biological Materials. The peptides angiotensin II, [Val^5]angiotensin II, random polymer Glu,Tyr (4:1), and histone H1 were purchased from *Sigma Chemicals Ltd.*, St. Louis, USA, or from *Fluka AG*. EGF was obtained from *Biomedical Technologies Inc.*, Stoughton, USA. [γ-^{32}P]ATP was from *Amersham*. A431 human squamous carcinoma cells were obtained from the *European Collection of Animal Cell Cultures* (*ECACC*), Porton Down, UK. The EGF-dependent BALB/MK mouse keratinocyte cell line was kindly provided by Dr. *S. Aaronson* [21]. Cell-culture reagents and materials were from *Gibco/BRL*, and tissue-culture plates were from *Falcon*.

4-[(E)-2-Nitroethenyl]phenol (**3**). Following [13], 4-hydroxybenzaldehyde (**1**; 122.12 g, 1 mol), nitromethane (214 ml, 4 mol), and freshly distilled aniline (10 ml, 0.11 mol) were heated to 80° for 6 h. A biphasic mixture of H_2O and $MeNO_2$ was then distilled from the mixture under vigorous stirring. Due to repeated bumping, the solvent was not evaporated to dryness, but the pulp suction filtered, leading to orange crystals and a dark brown mother liquor. The crystals were dissolved in 1.5 l of AcOEt and the aniline washed out with 150 ml of 2N HCl. The solvent was evaporated, the orange crystalline residue dissolved in 1 l of AcOEt, extracted with 2 × 100 ml of 2N HCl, and the org. layer dried and evaporated. The resulting crude orange-yellow crystals were recrystallized from MeOH/H_2O 1:1: 114 g (69%) of fine yellow needles. M.p. 164–165° ([13]: 167–168°). *Caution*: These crystals are strongly irritating for the nasal mucous membranes and were handled with respiratory filter only! IR: 3380 (OH), 3120

(arom. CH), no aliph. CH, no C=O, 1600 (C=C), 1515 (NO_2), 1485, 1435, 1330, 1280, 1255, 1195, 1165, 975, 965, 825. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): 10.42 (*s*, OH); 8.06 (*s*, CH=CHNO$_2$); 7.72 (*d*, *J* = 8.7, H–C(3), H–C(5)); 6.86 (*d*, *J* = 8.5, H–C(2), H–C(6)). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): 162.1 (*s*, C(1)); 140.3, 135.3 (2*d*, *C*H=*C*HNO$_2$): 132.7 (*d*, C(3), C(5)); 121.5 (*s*, C(4)); 116.6 (*d*, C(2), C(6)). EI-MS (70 eV): 165 (42, M^+), 148 (5), 118 (100, $[M - HNO_2]^+$), 107 (26), 91 (40), 77 (14), 65 (54), 51 (18), 39 (36). Anal. calc. for $C_8H_7NO_3$ (165.15): C 58.18, H 4.27, N 8.48; found: C 58.38, H 4.05, N 8.28.

*Methyl 4-[(*E*)-2-Nitroethenyl]phenyl Glutarate* (**8**). Following [14], TfOH (50 µl, 0.6 mmol) was added under Ar to a soln. of **3** (5.00 g, 30.3 mmol) in 45 ml of abs. $CHCl_3$ which led to immediate formation of black lumps. Then O^5-methyl glutar-1-yl chloride (**5**; 4.2 ml, 30.3 mmol) was added and the mixture stirred at r.t. for 10 h (yellow→dark brown). After addition of 125 ml of $CHCl_3$, the org. phase was washed successively with 125 ml of H_2O and 50 ml of sat. $NaHCO_3$ soln. then dried, and evaporated. The crude yellow **8** obtained (8.69 g, 98%) was recrystallized from 200 ml of MeOH: 5.80 g (65%) of yellow needles, after drying *in vacuo*. M.p. 117–118°. IR: 3110*w* (arom. CH), 2950*w* (aliph. CH), 1755*s* (C=O, phenyl ester), 1730*s* (C=O, methyl ester), 1630, 1600*w*, 1495*s* (NO_2), 1340*s*, 1280, 1260, 1210, 1170, 1135, 980, 965, 960, 925, 840 (*p*-disubst. Ar). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.99 (*d*, *J* = 13.6, 1 H, CH=CHNO$_2$); 7.57 (*d*, *J* = 8.6, H–C(3′), H–C(5′)); 7.56 (*d*, *J* = 13.7, 1 H, CH=CHNO$_2$); 7.21 (*d*, *J* = 8.7, H–C(2′), H–C(6′)); 3.70 (*s*, MeO); 2.68 (*t*, *J* = 7.3, CH_2(2)); 2.48 (*t*, *J* = 7.2, CH_2(4)); 2.09 (*quint.*, *J* = 7.2, CH_2(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.2* (*s*, C(5)); 170.9* (*s*, C(1)); 153.5 (*s*, C(1′)); 138.0, 137.2 (2*d*, *C*H=*C*HNO$_2$); 130.4 (*d*, C(3′), C(5′)); 127.7 (*s*, C(4′)); 122.7 (*d*, C(2′), C(6′)); 51.7 (*q*, MeO); 33.3, 32.9 (2*t*, C(2), C(4)); 19.9 (*t*, C(3)). EI-MS (70 eV): no M^+, 262 (10, $[M$ – MeO$]^+$), 234 (2), 129 (100, $[M$ – methyl glutarate$]^+$), 118 (6), 101 (50), 97 (10), 89 (6), 59 (46), 55 (25). CI-MS (NH_3): 312 (16), 311 (100, $[M + NH_4]^+$), 281 (12), 264 (24), 183 (4), 164 (6), 163 (11), 150 (10), 146 (31), 136 (6), 131 (37), 114 (6), 101 (10), 46 (15). Anal. calc. for $C_{14}H_{15}NO_6$ (293.28): C 57.34, H 5.16, N 4.78; found: C 57.17, H 5.11, N 4.95.

*Ethyl 4-[(*E*)-2-Nitroethenyl]phenyl Glutarate* (**9**). TfOH (30 µl, 0.3 mmol) and O^5-ethyl glutar-1-yl chlorid (**6**; 3.4 ml, 21.8 mmol) were added under Ar to a soln. of **3** (3.60 g, 21.8 mmol) in 45 ml of abs. CH_2Cl_2. The mixture was stirred at r.t. for 6.5 h until no more evolution of HCl was observed. The CH_2Cl_2 phase was successively washed with 50 ml of H_2O, 3 × 50 ml of sat. $NaHCO_3$ soln. and 2 × 50 ml of 2N NaOH, dried, and evaporated: 5.33 g (80%) of a yellow powder (pure by NMR). A sample was recystallized twice from boiling MeOH to yield yellow iridescent lamellac. M.p. 94–94.5°. UV (CH_2Cl_2): 318 (13400). IR: 3106, 3043*w* (arom. CH), 2983, 2943, 2915*w* (aliph. CH), 1744*s* and 1719*s* (C=O), 1638, 1601*w*, 1521, 1507*s*, 1342*s*, 1213, 1171, 1140, 841. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.98 (*d*, *J* = 13.7, 1 H, CH=CHNO$_2$); 7.58 (*d*, *J* = 8.4, H–C(3′), H–C(5′)); 7.56 (*d*, *J* = 13.7, 1 H, CH=CHNO$_2$); 7.21 (*d*, *J* = 8.5, H–C(2′), H–C(6′)); 4.16 (*q*, *J* = 7.1, CH_3CH_2O); 2.68 (*t*, *J* = 7.4, CH_2(2)); 2.46 (*t*, *J* = 7.2, CH_2(4)); 2.08 (*quint.*, *J* = 7.2, CH_2(3)); 1.27 (*t*, *J* = 7.1, CH_3CH_2O). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.1* (*s*, C(5)); 171.3* (*s*, C(1)); 153.7 (*s*, (C(1′)); 138.2, 137.3 (2*d*, *C*H=*C*HNO$_2$); 130.6 (*d*, C(3′), C(5′)); 127.8 (*s*, C(4′)); 122.8 (*d*, C(2′), C(6′)); 60.4 (*t*, CH_3CH_2O); 33.1, 32.9 (*t*, C(2), C(4)); 19.7 (*t*, C(3)); 13.9 (*q*, CH_3CH_2O). EI-MS (70 eV): no M^+, 262 (19, $[M$ – EtO$]^+$), 234 (3), 143 (100, $[M$ – **3**$]^+$), 115 (41), 97 (6), 87 (35), 55 (19), 43 (12), 42 (13). CI-MS (NH_3): 325 (100, $[M + NH_4]^+$), 308 (10, $[M + H]^+$), 295 (37), 293 (28), 279 (13), 278 (77), 178 (12), 160 (52), 136 (30), 131 (25), 114 (33). Anal. calc. for $C_{15}H_{17}NO_6$ (307.30): C 58.63, H 5.58, N 4.56; found: C 58.40, H 5.51, N 4.23.

*4-[(*E*)-2-Nitroethenyl]phenyl Hydrogen Glutarate* (**11**). A suspension of **3** (16.52 g, 0.1 mol), glutaric anhydride (7; 11.41 g, 0.1 mol), and $(Me_2N)C_5H_4N$ (0.12 g, 1 mmol) in 250 ml of abs. $CHCl_3$ was stirred at r.t. for 26 h and then cooled to 0°. The supernatant light yellow crystals were suction filtered, washed with a small amount of cold $CHCl_3$ and pentane and dried *in vacuo*. The resulting crude product (25.28 g (91%) of light yellow powder) consisted, according to ^{1}H-NMR, of **3** (8%), **11** (86%), **7** (4%), and glutaric acid (2%). The crude product (25.02 g) was dissolved in MeOH (500 ml) and the hot soln. filtered to remove a small amount of colorless crystals (glutaric acid). The filtrate was concentrated *in vacuo* to *ca.* 180 ml and warmed to 70° to dissolve all crystals. The soln. was left overnight at 4°, the precipitate suction filtered, washed with MeOH and dried *in vacuo*: 18.50 g (74% of the amount used for recrystallization, overall yield 68%) of short, yellow needles. M.p. 146.5–148°. ^{1}H-NMR: 94% pure, containing **3** (2.4%) and **7** (3.6%). Because **11** decomposes autocatalytically on silica gel due to its acid function, purification by column chromatography was not attempted. IR: 3300–2400 (br., COOH), 3105, 3040*w* (arom. CH), 2960, 2920*w* (aliph. CH), 1755*s* (phenyl ester), 1710*s* (COOH), 1630, 1600, 1580, 1520, 1495, 1420, 1385, 1335, 1285, 1220, 1175, 1130, 965, 830. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): 12.19 (br. *s*, OH); 8.24 (*d*, *J* = 13.7, 1 H, CH=CHNO$_2$); 8.16 (*d*, *J* = 13.7, 1 H, CH=CHNO$_2$); 7.93 (*d*, *J* = 8.6, H–C(3′), H–C(5′)); 7.28 (*d*, *J* = 8.6, H–C(2′), H–C(6′)); 2.66 (*t*, *J* = 7.3, CH_2(2)); 2.36 (*t*, *J* = 7.3, CH_2(4)); 1.87 (*quint.*, *J* = 7.3, CH_2(3)). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): 174.0 (*s*, C(5)), 171.1 (*s*, C(1)); 153.2 (*s*, C(1′)); 138.3, 138.0 (2*d*, *C*H=*C*HNO$_2$); 131.2 (*d*, C(3′), C(5′)); 127.9 (*s*, C(4′)); 122.7 (*d*, C(2′), C(6′)); 32.8, 32.6 (2*t*, C(2), C(4)); 19.7 (*t*, C(3)). FAB-MS (NBA): very weak signals, 281 (14), 280 (67, $[M + H]^+$), 279 (36, M^+), 273 (11), 262 (15, $[M$ – OH$]^+$), 246 (45), 167 (18), 166 (88,

$[3 + H]^+$), 165 (31, $[3]^+$), 161 (14), 150 (23), 149 (51), 139 (23), 137 (20), 134 (10), 123 (12), 119 (15), 115 (100), 101 (30), 93 (84).

2′,3′-O-Isopropylideneadenosine 5′-{4-[(E)-2-Nitroethenyl]phenyl Glutarate} (**14**). Following [11], a soln. of oxalyl chloride (1.5 ml, 17.9 mmol) and DMF (30 µl, 0.36 mmol) in 15 ml of abs. $CHCl_3$ was added dropwise under Ar at 0° within 15 min to a suspension of **11** (1.00 g, 3.6 mmol) in 10 ml of abs. $CHCl_3$. The flask was purged with Ar, closed with a $CaCl_2$ drying tube, the ice-bath removed, and the mixture stirred for 2 h. Evaporation yielded 1.23 g (116%) of a yellow, moist powder, which was used directly in the following experiment. IR (film of the reaction mixture): no acid OH, 3015 (arom. CH), 2975, 2870 (aliph. CH), 1780*s* (br., C=O), 1635*w* (C=C), 1600*w*, 1520, 1505, 1340, 1215*s*, 1165, 1130, 1110, 755*s* (br.), 660.

To a soln. of 2′,3′-*O*-isopropylideneadenosine (**13**; 1.11 g, 3.6 mmol) and $(Me_2N)C_5H_4N$ (8.6 mg, 0.07 mmol) in 5 ml of abs. DMF, a soln. of the acyl chloride in 8 ml of DMF was added dropwise under Ar within 25 min. The flask was purged with Ar, closed with a $CaCl_2$ drying tube, and the mixture stirred at r.t. for 3 h. The resulting dark yellow, clear soln. was evaporated at $\leq 52°/0.2$ mbar (20 min) to a viscous yellow oil, which dissolved completely upon addition of 100 ml of H_2O and 100 ml of AcOEt. The org. phase was washed with 25 ml of sat. NH_4Cl soln., dried, and evaporated yielding 1.82 g (89%) of yellowish crude product. This was dissolved in 100 ml of $CHCl_3$ and washed with 50 ml of sat. $NaHCO_3$ soln. mixed with plenty of crushed ice. After phase separation with NaCl, the org. layer was dried and evaporated: 1.59 g of yellow product. Flash chromatography (200 g of *Uetikon* silica gel, AcOEt/MeOH 100:0→90:10) afforded 776.5 mg of a yellow material. ^{1}H-NMR: **13** still present; **14** had partly decomposed on the *Uetikon* silica gel. Upon 2D TLC (*Merck* silica gel plates, 10 cm × 10 cm, AcOEt/pentane 9:1), **14** showed no signs of decomposition. The product mixture was, therefore, rechromatographed on *Merck* silica gel (125 g, AcOEt/pentane 9:1, then AcOEt/MeOH 100:0→90:10): 204.1 mg (10%) of **14**. Yellow, amorphous solid. M.p. 74–85°. IR: 3360 (br., NH_2), 3110*w* (arom. CH), 2990, 2940*w* (aliph. CH), 1740*s* (br., C=O), 1690*s*, 1635, 1605, 1515, 1505, 1470, 1415, 1380, 1340, 1210, 1170, 970, 865, 840. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.32 (*s*, H–C(8″)); 7.93 (*s*, H–C(2″)); 7.91 (*d*, *J* = 12.5, 1 H, CH=CHNO$_2$); 7.54 (*d*, *J* = 13.6, 1 H, CH=CHNO$_2$); 7.51 (*d*, *J* = 8.5, H–C(3‴), H–C(5‴)); 7.14 (*d*, *J* = 8.7, H–C(2‴), H–C(6‴)); 6.79 (*s*, NH_2); 6.14 (*d*, *J* = 1.7, H–C(1′)); 5.52 (*dd*, *J* = 6.2, 1.8, H–C(2′)); 5.10 (*dd*, *J* = 6.2, 3.4, H–C(3′)); 4.50 (*m*, H–C(4′)); 4.41 (*dd*, *J* = 11.8, 4.4, 1 H, H–C(5′)); 4.29 (*dd*, *J* = 11.8, 6.3, 1 H, H–C(5′)); 2.61 (*t*, *J* = 7.4, CH_2(2)); 2.40 (*m*, CH_2(4)); 2.00 (*quint.*, *J* = 7.1, CH_2(3)); 1.62, 1.40 (2*s*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.3 (*s*, C(5)); 170.9 (*s*, C(1)); 156.0 (*s*, C(6″)); 153.4 (*s*, C(1‴)); 153.1 (*d*, C(2″)); 149.1 (*s*, C(4″)); 139.7 (*s*, C(8″)); 137.9, 137.1 (2*d*, *C*H=*C*HNO$_2$); 130.4 (*d*, C(3‴), C(5‴)); 127.6 (*s*, C(4‴)); 122.6 (*d*, C(2‴), C(6‴)); 120.1 (*s*, C(5″)); 114.5 (*s*, Me_2C); 91.0 (*d*, C(1′)); 84.9, 84.1, 81.7 (3*d*, C(2′), C(3′), C(4′)); 64.1 (*t*, C(5′)); 33.1, 32.7 (2*t*, C(2), C(4)); 27.1, 25.4 (2*q*, *Me*$_2$C); 19.7 (*t*, C(3)). FAB-MS (NBA): 570 (30), 569 (100, $[M + H]^+$), 404 (19), 164 (15), 97 (18), 69 (15), 59 (13), 55 (37), 43 (20), 41 (17). HR-FAB-MS (NBA): 568.191 ● 0.0015 ($C_{26}H_{28}N_6O_9$, calc. 568.1918).

Adenosine 5′-{4[(E)-2-Nitroethenyl]phenyl Glutarate} (**16**). A soln. of **14** (218 mg, 0.38 mmol) and 4 ml of 1N HCl in 10 ml of acetone was stirred at r.t. for 22 h. The mixture was neutralized with 30 ml of phosphate buffer (pH 6.8, *c* = 0.5M) to give pH 5.95. The orange yellow slurry was frozen in liq. N_2 and lyophilized at $0°/\geq 0.04$ mbar to give a yellow powder, to which 100 ml of AcOEt were added. The mixture was washed with 2 × 50 ml of H_2O and 10 ml of sat. NH_4Cl soln., the combined aq. phase reextracted with 2 × 25 ml of AcOEt, the org. phase dried and evaporated, and the crude product (141 mg of a yellow film) separated by conventional chromatography (30 g of *Merck* silica gel, CH_2Cl_2/MeOH 9:1): 62 mg (31%) of **16**. Yellow, viscous honey with no sharp m.p. IR: 3600–2500*s* (br., OH), 1730, 1710*s* (br., C=O), 1605*s* (C=C), 1505, 1495*s*, 1420, 1380, 1340, 1260, 1215, 1170, 1130, 1075, 975, 890, 855, 835, 775, 720, 700. ^{1}H-NMR (300 MHz, $(CD_3)_2CO$)[2]: 8.29, 8.28 (2*s*, H–C(2″), H–C(8″)); 8.01 (*d*, *J* = 13.7, 1 H, CH=CHNO$_2$); 7.96 (*d*, *J* = 13.6, 1 H, CH=CHNO$_2$); 7.88 (*d*, *J* = 8.8, H–C(3‴), H–C(5‴)); 7.28 (*d*, *J* = 8.8, H–C(2‴), H–C(6‴)); 7.24 (*m*, NH_2); 6.07 (*d*, *J* = 4.3, H–C(1′)), 4.87 (*t*, *J* = 4.8, H–C(2′)); 4.55 (*t*, *J* = 5.1, H–C(3′)); 4.45 (*dd*, *J* = 11.9, 3.7, 1 H, H–C(5′)); 4.36 (*dd*, *J* = 11.9, 5.2, 1 H, H–C(5′)); 4.28 (*m*, H–C(4′)); 3.8–2.8 (br., OH–C(2′), OH–C(3′)); 2.70 (*t*, *J* = 7.3, CH_2(2)); 2.52 (*t*, *J* = 7.3, CH_2(4)); 2.00 (*quint.*, *J* = 7.3, CH_2(3)). ^{13}C-NMR (101 MHz, $(CD_3)_2CO$); 173.1* (C(5)); 171.8* (C(1)); 156.1 (C(6″)); 154.6 (C(1‴)); 151.5 (C(2″)); 150.4 (C(4″)); 141.2 (C(8″)); 138.8, 138.6 (*C*H=*C*HNO$_2$); 131.7 (C(3‴), C(5‴)); 129.0 (C(4‴)); 123.7 (C(2‴), C(6‴)); 120.7 (C(5″)); 90.0 (C(1′)); 83.1 (C(4′)); 74.9 (C(2′)); 71.8 (C(3′)); 64.6 (C(5′)); 33.6, 33.4 (C(2), C(4)); 20.7 (C(3)). HR-FAB-MS (NBA): 528.160 ± 0.0008 ($C_{23}H_{24}N_6O_9$, calc. 528.1605).

2′,3′-O-Isopropylideneadenosine 5′-{Methyl Glutarate} (**17**). A mixture of **13** (0.94 g, 3.1 mmol), $(Me_2N)C_5H_4N$ (0.35 g, 2.8 mmol), O^5-methyl glutar-1-yl chloride (**5**; 0.56 g, 3.4 mmol) and 20 ml of abs. $CHCl_3$

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[2]) Compound **16** was insoluble in $CHCl_3$, thus the NMR spectra were recorded in $(CD_3)_2CO$ where gelatinization was observed at concentrations higher than *ca.* 5 mg/ml.

was refluxed for 17 h, cooled, washed successively with 25 ml of H_2O, 2 × 10 ml of 2N HCl, and 10 ml of sat. NH_4Cl soln., dried, and evaporated. The resulting colorless oil (^{1}H-NMR: several products) was dissolved in 10 ml of $CHCl_3$, washed with 10 ml of sat. $NaHCO_3$ soln., dried (Na_2SO_4), and evaporated. The colorless, opaque, viscous oil (437 mg) was chromatographed (*Merck* silica-gel column, *Lobar* size *A*; 6 bar overpressure, AcOEt/acetone 1:1): 253 mg (15%) of **18** and 55 mg (4%) of **17**, each as a white viscous gum. **17**: ^{1}H-NMR (300 MHz, $CDCl_3$): 8.35 (*s*, H–C(8″)); 7.90 (*s*, H–C(2″)); 6.11 (*s*, H–C(1′), NH_2); 5.50 (*dd*, *J* = 6.3, 1.7, H–C(2′)); 5.07 (*dd*, *J* = 6.3, 3.3, H–C(3′)); 4.48 (*m*, H–C(4′)); 4.37 (*dd*, *J* = 11.8, 4.5, 1 H, H–C(5′)); 4.24 (*dd*, *J* = 11.8, 6.3, 1 H, H–C(5′)); 3.66 (*s*, MeO); 2.34, 2.32, (2*t*, *J* = 7.3, CH_2(2), CH_2(4)); 1.90 (*quint.*, *J* = 7.3, CH_2(3)); 1.62, 1.41 (2*s*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.3, 172.4 (2*s*, C(1), C(5)); 155.7 (*s*, C(6″)); 153.2 (*d*, C(2″)); 149.3 (*s*, C(4″)); 139.7 (*d*, C(8″)); 120.3 (*s*, C(5″)); 114.6 (*s*, Me_2C); 91.0 (*d*, C(1′)); 84.9, 84.2, 81.7 (3*d*, C(2′), C(3′), C(4′)); 64.0 (*t*, C(5′)); 51.6 (*q*, MeO); 32.9 (*t*, C(2), C(4)); 27.2, 25.4 (2*q*, Me_2C); 19.9 (*t*, C(3)). **18**: ^{1}H-NMR (300 MHz, $CDCl_3$): 9.76 (*s*, NH); 8.71* (*s*, H–C(2″)); 8.30* (*s*, H–C(8″)); 6.21 (*d*, *J* = 1.9, H–C(1′)); 5.50 (*dd*, *J* = 6.3, 1.9, H–C(2′)); 5.09 (*dd*, *J* = 6.3, 3.3, H–C(3′)); 4.51 (*m*, H–C(4′)); 4.36 (*dd*, *J* = 11.8, 4.6, 1 H, H–C(5′)); 4.26 (*dd*, *J* = 11.9, 6.2, 1 H, H–C(5′)); 3.66, 3.65 (2*s*, 2 MeO); 3.05, 2.50, 2.33, 2.30 (4*t*, *J* = 7.3, 4 CH_2C=O); 2.11, 1.87 (2*quint.*, *J* = 7.3, 2 CH_2–CH_2–CH_2); 1.63, 1.41 (2*s*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.6, 173.2, 173.0, 172.3 (4 C=O); 152.4, 150.8 (C(6″), C(2″)); 149.6 (C(4″)); 142.6 (C(8″)); 122.6 (C(5″)); 114.7 (Me_2C); 91.1 (C(1′)); 85.0, 84.2, 81.7 (C(2′), C(3′), C(4′)); 63.9 (C(5′)); 51.6, 51.5 (2 MeO); 36.7, 33.1, 32.9, 32.9 (4 CH_2C=O); 27.1, 25.4 (Me_2C); 20.0, 19.9 (2 CH_2–CH_2–CH_2).

*2-Methoxy-4-[(*E*)-2-nitroethenyl]phenol* (**4**). In analogy to the synthesis of **3**, a soln. of vanilline (**2**; 1.52 g, 10 mmol) and aniline (0.10 ml, 1 mmol) in 5 ml of nitromethane (5.68 g, 93 mmol) was stirred at 70° for 24 h. The mixture was evaporated, the crude product dissolved in 50 ml of AcOEt, extracted with 3 × 60 ml of 2N HCl, the org. phase dried and evaporated, and the product recrystallized 3 times from MeOH/H_2O 1:1: 1.56 g (80%) of bright yellow crystals. M.p. 172.5–174.5°. UV (EtOH): 206 (23320), 240 (sh), 258 (7520), 376 (16280). IR: 3495*s* (OH), 3140 (arom. CH), no C=O, 1635*w*, 1615*s*, 1525, 1495*s*, 1480, 1440, 1370*s*, 1305, 1260, 1220, 1200, 1170, 1140, 1030, 980, 960, 860, 825. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): 10.04 (*s*, OH); 8.15, 8.03 (2*d*, *J* = 13.4, CH=CHNO$_2$); 7.48 (*d*, *J* = 1.9, H–C(3)); 7.30 (*dd*, *J* = 8.2, 1.9, H–C(5)); 6.86 (*d*, *J* = 8.2, H–C(6)); 3.84 (*s*, MeO). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): 151.2* (*s*, C(2)); 148.1* (*s*, C(1)); 140.2, 135.0 (2*d*, *C*H=*C*HNO$_2$); 125.8 (*d*, C(5)); 121.5 (*s*, C(4)); 115.7* (*d*, C(6)); 112.2* (*d*, C(3)); 56.0 (*q*, MeO). FAB-MS (NBA): 196 (39, $[M + H]^+$), 195 (27, M^+), 180 (6, $[M - CH_3]^+$), 166 (8). Anal. calc. for $C_9H_9NO_4$ (195.17): C 55.39, H 4.65, N 7.18; found: C 55.30, H 4.65, N 6.64.

*2-Methoxy-4-[(*E*)-2-nitroethenyl]phenyl Hydrogen Glutarate* (**12**). To a soln. of **4** (19.64 g, 100 mmol) in 500 ml of abs. CH_2Cl_2, **7** (11.4 g, 100 mmol; recrystallized from abs. $CHCl_3$/pentane 1:1) and $(Me_2N)C_5H_4N$ (1.13 g, 10 mmol) were added. The mixture was stirred at r.t. for 69 h (reddish→yellow), then cooled, and suction filtered. The crystals obtained were dissolved in 2 l of AcOEt washed successively with 3 × 150 ml of 2N HCl and 3 × 100 ml of sat. NH_4Cl soln., and the org. phase was dried and evaporated. Recrystallization from MeOH yielded 18.42 g (60%) of shining, yellow needles. M.p. 164.0–165.5°. UV (EtOH): 249 (8760), 305 (sh), 332 (12120). IR: 3110, 2940*w* (aliph. CH), 1760*s* (C=O, phenyl ester), 1685*s* (br., C=O, acid), 1630, 1595*s* (br., C=C), 1580, 1500*s* (NO_2), 1440, 1410, 1345*s* (NO_2), 1300, 1265, 1240, 1175, 1165, 1115*s* (br.), 1025, 970, 825, 710. ^{1}H-NMR (300 MHz, $(CD_3)_2CO$): 10.68 (br. *s*, OH); 8.10, 8.03 (2*d*, *J* = 13.7, CH=CHNO$_2$); 7.63 (*d*, *J* = 1.8, H–C(3′)); 7.43 (*dd*, *J* = 8.1, 1.9, H–C(5′)); 7.23 (*d*, *J* = 8.2, H–C(6′)); 3.92 (*s*, MeO); 2.71 (*t*, *J* = 7.3, CH_2(2)); 2.50 (*t*, *J* = CH_2(4)); 2.01 (*quint.*, *J* = 7.3, CH_2(3)). ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): 12.15 (*s*, OH); 8.31 (*d*, *J* = 13.6, 1 H, CH=CHNO$_2$); 8.12 (*d*, *J* = 13.7, 1 H, CH=CHNO$_2$); 7.67 (*d*, *J* = 1.4, H–C(3′)); 7.46 (*dd*, *J* = 8.2, 1.6, H–C(5′)); 7.22 (*d*, *J* = 8.0, H–C(6′)); 3.84 (*s*, MeO); 2.64 (*t*, *J* = 7.2, CH_2(2)); 2.37 (*t*, *J* = 7.2, CH_2(4)); 1.86 (*quint.*, *J* = 7.1, CH_2(3)). ^{13}C-NMR (75 MHz, $(CD_3)_2CO$): 174.8 (*s*, C(5)); 171.9 (*s*, C(1)); 153.4 (*s*, C(2′)); 144.5 (*s*, C(1′)); 139.7, 139.2 (2*d*, *C*H=*C*HNO$_2$); 130.7 (*s*, C(4′)); 125.0, 124.6 (2*d*, C(5′), C(6′)); 113.8 (*d*, C(3′)); 56.8 (*q*, MeO); 33.5, 33.0 (2*t*, C(2), C(4)); 21.1 (*t*, C(3)). Anal. calc. for $C_{14}H_{15}NO_7$ (309.28): C 54.37, H 4.89, N 4.53; found: C 54.13, H 4.76, N 4.71.

*2-Methoxy-4-[(*E*)-nitroethenyl]phenyl Methyl Glutarate* (**10**). To a soln. of **12** (5.00 g, 16.2 mmol) in 100 ml of MeOH, 120 ml of a diazomethane/Et_2O soln. (containing *ca.* 1.5 g (36 mmol) of CH_2N_2) were added at 0° until no further gas evolution was observed. The mixture was left at 0° for 1 h and r.t. for 1 h and then evaporated. The crude product was dissolved in AcOEt and washed with 4 × 50 ml of 2N HCl and 3 × 50 ml of sat. $NaHCO_3$ soln. The org. layer was dried and evaporated: 1.75 g (33%) of **10**. Light yellow crystals. M.p. 93.0–96.5°. UV (EtOH): 247 (12080), 305 (sh), 332 (16840). IR: 3130 (arom. CH), 2955 (aliph. CH), 1770*s* (C=O, phenyl ester), 1730*s* (C=O, methyl ester), 1630*w*, 1595, 1495, 1420, 1400, 1360, 1330, 1280, 1265, 1215, 1170, 1125*s*, 1080, 1035, 995. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): 8.32 (*d*, *J* = 13.6, 1 H, CH=CHNO$_2$); 8.13 (*d*, *J* = 13.6, 1 H, CH=CHNO$_2$); 7.68 (*d*, *J* = 1.8, H–C(3′)); 7.47 (*dd*, *J* = 8.2, 1.8, H–C(5′)); 7.23 (*d*, *J* = 8.2, H–C(6′)); 3.84 (*s*, MeO–C(2′)); 3.63 (*s*, COOMe); 2.65 (*t*, *J* = 7.4, CH_2(2)); 2.46 (*t*, *J* = 7.4, CH_2(4)); 1.90 (*quint.*, *J* = 7.3, CH_2(3)). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): 173.4 (*s*, C(5)); 171.0 (*s*, C(1)); 151.7 (*s*, C(2′)); 142.7 (*s*, C(1′)); 139.2, 138.7 (2*d*, *C*H=*C*HNO$_2$); 129.7

(*s*, C(4′)); 124.1, 123.9 (2*d*, C(5′), C(6′)); 113.4 (*d*, C(3′)); 56.3 (*q*, *Me*O–C(2′)); 51.5 (*q*, COO*Me*); 32.3, 32.1 (2*t*, C(2), C(4)); 19.9 (*t*, C(3)). FAB-MS (NBA): 324 (33, $[M + H]^+$), 196 (30, $[\mathbf{4} + H]^+$), 129 (100). Anal. calc. for $C_{15}H_{17}NO_7$ (323.30): C 55.73, H 5.30, N 4.33, O 34.64; found: C 55.69, H 5.40, N 4.49, O 34.66.

*2′,3′-O-Isopropylideneadenosine 5′-{2-Methoxy-4-[(*E*)-2-nitroethenyl]phenyl Glutarate}* (**15**). A mixture of oxalyl chloride (1.4 ml, 15 mmol) and DMF (23 µl, 0.3 mmol) was added dropwise under Ar at 0° within 15 min to a soln. of **12** (0.93 g, 3 mmol) in 20 ml of abs. $CHCl_3$. The mixture was stirred at r.t. for 3 h and was then evaporated, yielding a yellow powder, which was used directly in the following reaction. IR (film of the reaction mixture): no acid OH, 3100*w* (arom. CH), 3000, 2960, 2920 (aliph. CH), 1775, 1750*s* (C=O), 1620, 1588, 1492, 1450, 1405, 1335, 1290, 1260, 1207, 1150, 1117, 1023, 957, 750*s* (br.), 660.

To a soln. of **13** (0.92 g, 3 mmol) and $(Me_2N)C_5H_4N$ (0.37 g, 3 mmol) in 20 ml of abs. $CHCl_3$, a soln. of the acyl chloride was added dropwise under Ar at r.t. within 15 min. After stirring at r.t. for 72 h the orange-yellow mixture was diluted with 150 ml of $CHCl_3$, washed with 50 ml of H_2O, 3 × 50 ml of 2N HCl, and 3 × 50 ml of sat. NH_4Cl soln., dried, and evaporated. The yellow-orange crude product (1.3 g) was purified by column chromatography (500 g silica gel, CH_2Cl_2/MeOH 96:4): 226 mg (13%) of yellow **15**. M.p. 63.5–68°[3]. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.33, 7.91 (2*s*, H–C(2″), H–C(8″)); 7.91, 7.54 (2*d*, *J* = 13.7, CH=CHNO$_2$); 7.13–7.04 (*m*, H–C(3‴), H–C(5‴), H–C(6‴)); 6.49 (*s*, NH_2); 6.12 (*d*, *J* = 1.9, H–C(1′)); 5.52 (*dd*, *J* = 6.3, 1.7, H–C(2′)); 5.09 (*dd*, *J* = 6.2, 3.4, H–C(3′)); 4.50 (*m*, H–C(4′)); 4.40 (*dd*, *J* = 11.8, 4.5, 1 H, H–C(5′)); 4.28 (*dd*, *J* = 11.8, 6.3, 1 H, H–C(5′)); 3.80 (*s*, MeO); 2.63 (*t*, *J* = 7.3, CH_2(2)); 2.43 (*m*, CH_2(4)); 2.03 (*quint.*, *J* = 7.3, CH_2(3)); 1.62, 1.40 (2*s*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.3 (*s*, C(5)); 170.5 (*s*, C(1)); 155.9 (*s*, C(6″)); 153.2 (*d*, C(2″)); 151.7 (*s*, C(2‴)); 149.2 (*s*, C(4″)); 142.9 (*s*, C(1‴)); 139.7 (*d*, C(8″)); 138.4 (137.2 (2*d*, *C*H=*C*HNO$_2$); 128.9 (*s*, C(4‴)); 123.7, 122.5 (2*d*, C(5‴), C(6‴)); 120.2 (*s*, C(5″)); 114.6 (*s*, Me_2C); 112.1 (*d*, C(3‴)); 91.0 (*d*, C(1′)); 84.9, 84.2, 81.7 (3*d*, C(2′), C(3′), C(4′)); 64.1 (*t*, C(5′)); 56.0 (*q*, MeO); 32.9, 32.7 (2*t*, C(2), C(4)); 27.2, 25.4 (2*q*, Me_2C); 20.0 (*t*, C(3)). FAB-MS (NBA): 600 (32), 599 (100, $[M + H]^+$), 404 (33), 290 (13), 164 (13), 97 (17), 73 (10), 69 (12), 55 (47), 43 (15).

Preparation of Enzymes. Partially purified EGF-R kinase was prepared from A431 cells as previously described [16] [17]. Recombinant *v-abl* kinase was expressed in *E. coli* using vector pablHP. The protein A-fusion product encoded by this vector was affinity-purified on IgG-Affigel as previously described [22]. Recombinant *c-src* was expressed in Sf9 cells using recombinant baculovirus and purified as described [18]. PKC from porcine brain was prepared as previously described [19].

Kinase Assays. Determination of EGF-R kinase activity was performed as previously described [12] [17] [19], using A431 membranes as the enzyme source and [γ-^{32}P]ATP (15 µM, 0.2 mCi/mmol) and angiotensin II (1 mg/ml) as substrates. All compounds were dissolved in Me_2SO and diluted with H_2O giving a final concentration of 1% in the assay. Genistein (**20**) and erbstatin (**19**) served as internal standards. *V-abl* kinase was assayed as previously described [12] [17] [22], using [Val^5]angiotensin II (1 mM) and [γ-^{32}P]ATP (10 mM, 0.33 mCi/mmol) as substrates. *C-src* tyrosine kinase activity was assayed as previously described [17] [19], using the random polymer Glu,Tyr (4:1) (25 µg/ml) and [γ-^{32}P]ATP (20 µM, 0.1 mCi/mmol) as substrates. Activity determinations of PKC using histone III-S (0.2 mg/ml) as substrate was performed as described previously [19]. IC_{50} values were defined as the inhibitor concentrations that resulted in a 50% inhibition of substrate phosphorylation by the enzyme compared to the control experiment in the absence of inhibitor.

Antiproliferative Assays. Assays with EGF-dependent BALB/MK mouse epidermal keratinocytes were performed essentially as described previously [12] [17] [19]. IC_{50} values were defined as the inhibitor concentrations that resulted in a 50% decrease in cell number as compared to the control cultures in the absence of inhibitor.

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[3]) This product contained, according to ^{13}C-NMR, 13% of **4**.

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