50. Synthesis of Modified Tripeptides and Tetrapeptides as Potential Bisubstrate Inhibitors of the Epidermal Growth Factor Receptor Protein Tyrosine Kinase

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The synthesis of a series of bisubstrate inhibitors of the epidermal growth factor receptor protein kinase (EGF-R PTK) consisting of small peptides linked covalently to adenosine *via* appropriate triphosphate substitutes is described. Boc-Glu(O'Bu)-Tyr-Leu-OBzl (**5**) and Ac-Glu(O'Bu)-Tyr-Leu-Arg(Pmc)-NH_2 (**8**; Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl) were prepared by standard peptide chemistry, (*Scheme 1*), then modified at the OH group of tyrosine either with adipic anhydride or with 4-(chlorosulfonyl)benzoic acid, 4-(chlorosulfonyl)-2-hydroxybenzoic acid, or benzene-1,4-disulfonyldichloride (*Scheme 2*), and finally coupled with the 5'-OH group of 2',3'-*O*-isopropylideneadenosine (*Scheme 3*). In addition, N^6-[(benzyloxy)carbonyl]-2',3'-*O*-isopropylideneadenosine 5'-(hydrogenhexanedioate) (**26**), an ATP substitute, was coupled with the morpholide of **5** (*Scheme 4*). Removal of the protecting groups gave the bisubstrate analogs **23**, **24**, and **28**. The compounds synthesized were tested as inhibitors of the EGF-R PTK. The most active bisubstrate-type inhibitor was **24**, composed of the tripeptide sequence H-Glu-Tyr-Leu-OBzl, the 2-hydroxy-4-sulfonylbenzoyl moiety, and adenosine; it showed an IC_{50} value of 33 μM.

Introduction. – The proliferation of cells is controlled by complex signal transduction pathways. Regulatory extracellular signals are transduced across the cell membrane by transmembrane receptors and carried to the nucleus through complex reaction cascades, where they stimulate cellular functions. The phosphorylation of proteins on tyrosine residues by protein tyrosine kinases (PTKs) is of prime importance in such transduction pathways [1]. Many studies have established that enhanced activity of PTKs has been implicated in certain human malignant and nonmalignant proliferative diseases (*e.g.* cancer, psoriasis, restenosis, *etc.*) [2].

The receptor tyrosine kinases (RTKs) have intrinsic PTK activity and participate in transmembrane signaling [3]. These receptors are involved in the control of cellular differentiation programs and cell growth. The epidermal growth factor receptor (EGF-R) is a well-characterized member of the large PTK family. It consists of an extracellular binding domain connected trough a transmembrane domain to an intracellular domain which contains a protein tyrosine kinase region and a C-terminal tail [1] [4–8]. Binding of the epidermal growth factor (EGF) or the transforming growth factor α (TGF-α) to the EGF-R [4] [8] induces a conformational change which leads to receptor dimerization. This dimerization then enables mutual phosphorylation of the intracellular domain of the EGF-R ('autophosphorylation') and increases the enzymat-

ic activity of the EGF-R with respect to phosphorylation of cytoplasmic substrates [9]. Also, overexpression of the EGF-R was shown to be implicated in some types of human cancers (*e.g.* breast tumors) [1] [10]. Thus, compounds that selectively block the activity of EGF-R and, therefore, the signaling pathways could be potential drugs in the treatment of epithelial diseases. To date, several classes of inhibitors of tyrosine kinases have been synthesized [11–16], but none of these inhibitors take advantage of the peptide/protein substrate specificity. In our study, we report the synthesis and initial testings of series of potential bisubstrate analogs based on small peptides, using the intracellular domain of the EGF-R (EGF-R ICD) as the target.

Concept and Design of Inhibitors. – For the transfer of the γ-phosphate group of ATP to a tyrosine moiety in a substrate molecule, a transition state has been postulated with a pentacoordinated P(γ) atom and with the α- and β-phosphate groups complexed with two bivalent metal ions (usually Mg^{2+} or Mn^{2+}) and Arg^{817} (*Fig.*). As a guideline for the design of potential inhibitors of the epidermal growth factor receptor tyrosine kinase, we used a molecular model of the kinase domain of EGF-R constructed from crystallographic data of the cAMP-dependent protein kinase [16] [17]. On the basis of this model and our previous work [18–20], we designed series of bisubstrate inhibitors consisting of a tri- or tetrapeptide as the protein substrate substitute, a 4-sulfonylbenzoyl (**1**), a 2-hydroxy-4-sulfonylbenzoyl (**2**), a benzene-1,4-disulfonyl (**3**), or an adipoyl moiety (**4**) as the triphosphate mimic or spacer, and adenosine. The 4-sulfonylbenzoyl and dicarbonyl spacers have already been successfully used in the design of multisubstrate-type inhibitors [18–20]. The sequence Glu-Tyr-Leu[1]) used for the tripeptide moiety was derived from a consensus sequence for the phosphorylation site of natural substrates of EGF-R [21], whereas the tetrapeptide sequence Glu-Tyr-Leu-Arg corresponds to the major autophosphorylation site of the EGF-R [8]. Combinations of each of the two peptides with one of the triphosphate mimics/spacers led to series of bisubstrate-type inhibitors of EGF-R. In addition, the synthesis of a phosphotyrosine-containing peptide was investigated as well.

Syntheses. – The protected tripeptide **5** (*Scheme 1*) was synthesized in solution by a conventional peptide-synthesis method from commercially available Boc-Glu(O^{t}Bu)-OSu and the hydrochloride of H-Tyr-Leu-OBzl. For the preparation of the morpholide **6**, the benzyl ester **5** was converted to the corresponding acid by catalytic hydrogenation. The mixed anhydride of the latter was then treated with morpholine.

The protected tetrapeptide amide **8** (*Scheme 1*) was prepared by a combined solid-phase and solution method. First, the *N*-acetylated peptide acid **7** was built up by standard Fmoc methodology [22] on 4-[4-(hydroxymethyl)-3-methoxyphenoxy]butyric acid benzhydrylamine (HMPB-BHA) polystyrene resin [23] using *N*,*N*′-diisopropylcarbodiimide/1-hydroxy-1*H*-benzotriazol (DICD/HOBt) activation. Compound **7** was then

[1]) Symbols and abbreviations used for amino acids, peptides, and nucleosides are in accordance with IUPAC-IUB recommendations. Additional abbreviations: Boc, (*tert*-butoxy)carbonyl; ^{t}Bu, *tert*-butyl; Bzl, benzyl; Dmtr, 4,4′-dimethoxytriphenylmethyl; Fmoc, (9*H*-fluoren-9-ylmethoxy)carbonyl; HOBt, 1-hydroxy-1*H*-benzotriazole; HOSu, *N*-hydroxysuccinimide; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Z, (benzyloxy)carbonyl; A(>CMe_2) = 2′,3′-*O*-isopropylideneadenosine residue.

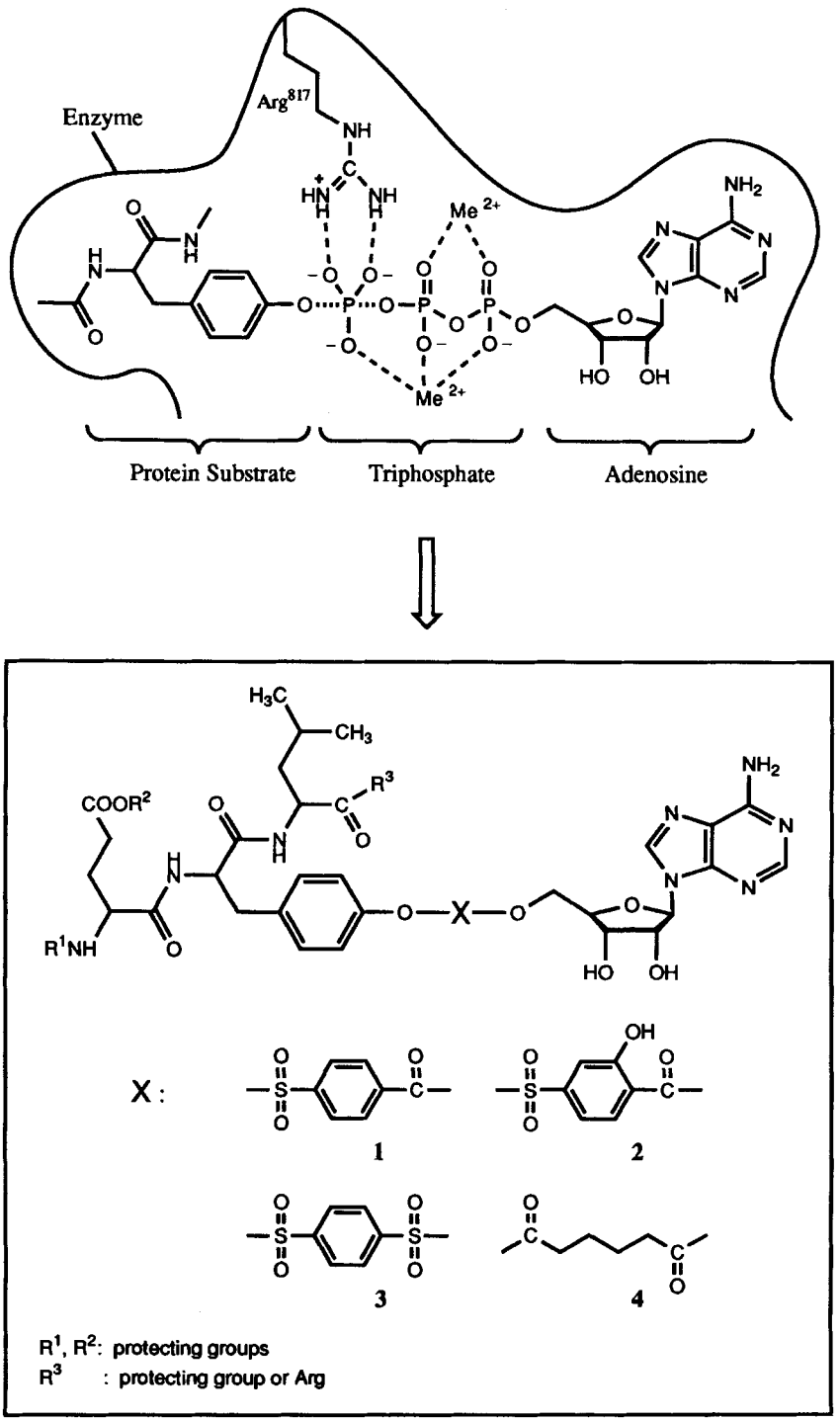
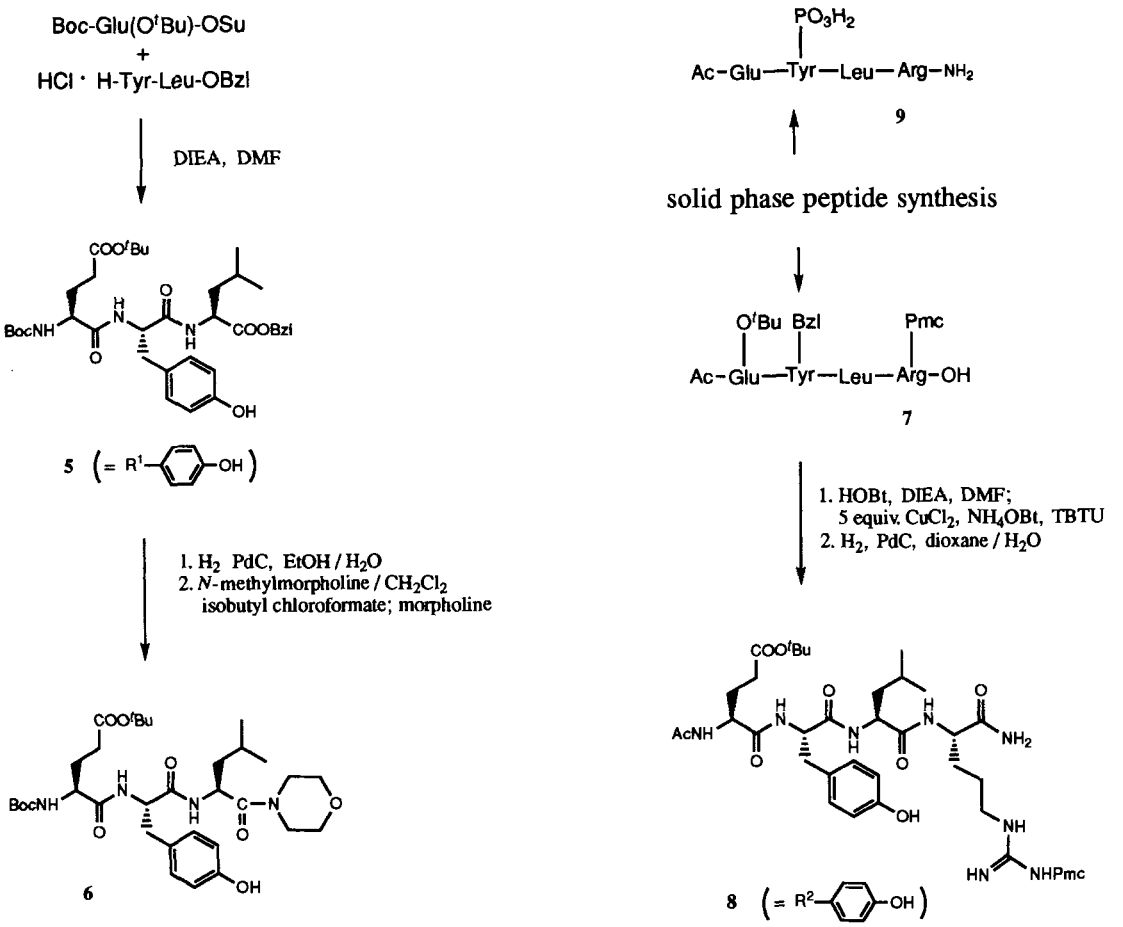


Figure. *Postulated transition state* (adapted from [17] [16]) *and schematic representation of possible bisubstrate inhibitors*

Scheme 1



DIEA = $(^i\text{Pr})_2\text{EtN}$

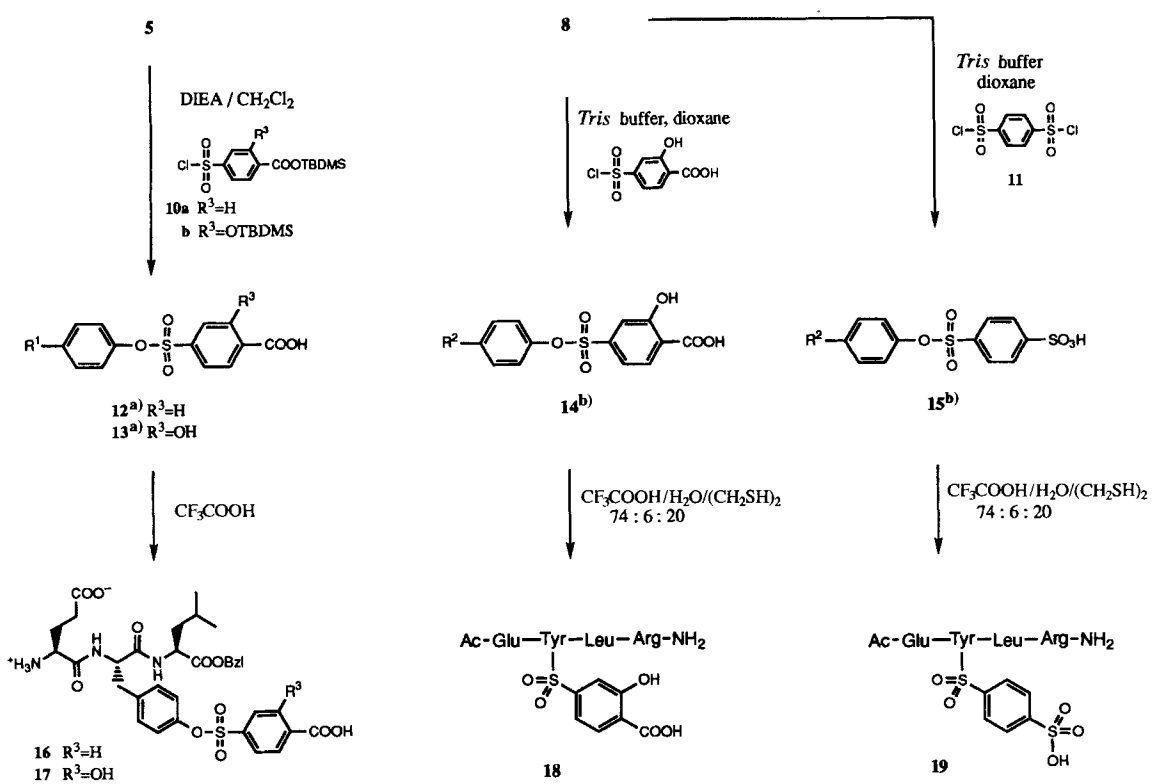
removed from the resin by selective acidolysis of the C-terminal ester bond with 1% trifluoroacetic acid (CF_3COOH) in CH_2Cl_2 and was converted to the amide by reaction with the ammonium salt of HOBt (NH_4OBt) using TBTU (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate)/HOBt [24] as condensating agent in the presence of *Hünig* base (*N*,*N*-diisopropylethylamine $(^i\text{Pr})_2\text{EtN}$) and of copper(II) chloride in dimethylformamide (DMF). The use of copper(II) ions eliminates virtually epimerization of a peptide's C-terminal residue [25] (*cf.* also [26–28]). The benzyl group was removed selectively by catalytic hydrogenation to yield **8**.

The phosphotyrosine-containing peptide amide **9** (*Scheme 1*) was prepared by Fmoc methodology [22] on 4-(2′,4′-dimethoxyphenyl-Fmoc-aminomethyl)phenoxyacetamido-MBHA polystyrene (*Rink* amide) resin (MBHA = 4-methylbenzhydrylamine). The synthetic strategy made use of TPTU (2-(2-oxo-1-pyridyl)-1,1,3,3-tetramethyluronium tetrafluoroborate)/HOBt [24] activation throughout, except for chain assembly of

Fmoc-Tyr(PO_3H_2)-OH (unprotected phosphate group [29]) where *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethaminium hexafluorophosphate *N*-oxide (HATU) [30] [31] was successfully used as activating agent. The free phosphotetrapeptide amide **9** was obtained after treatment of the resin with CF_3COOH/H_2O/ethane-1,2-dithiol($(CH_2SH)_2$) 74:6:20.

The 4-sulfonylbenzoyl derivatives **12** and **13** as well as **14** and the benzene-1,4-disulfonyl derivative **15** (*Scheme 2*) were prepared by reaction of the respective peptides with 4-(chlorosulfonyl)benzoic acid (for compound **12**), 4-(chlorosulfonyl)-2-hydroxybenzoic acid (for compounds **13** and **14**), or benzene-1,4-disulfonyl dichloride (**11**) [32] [33] (for compound **15**), under modified *Schotten-Baumann* conditions. However, compounds **12** and **13** could be obtained in higher yields when the (chlorosulfonyl)benzoic acids used were first silylated with *N*-methyl-*N*-[(*tert*-butyl)dimethylsilyl]trifluoroacetamide/(*tert*-butyl)dimethylsilyl chloride ($CF_3CON(Me)Si(^tBu)Me_2$/$^tBuMe_2SiCl$); the (*tert*-butyl)-dimethylsilyl derivatives **10a** and **10b** thus obtained were then each reacted *in situ* with tripeptide **5** in abs. CH_2Cl_2 in the presence of $(^iPr)_2EtN$. The free peptide-triphosphate analogs **16**–**19** were obtained after deprotection with CF_3COOH or CF_3COOH/H_2O/$(CH_2SH)_2$ 76:4:20 and precipitation from Et_2O.

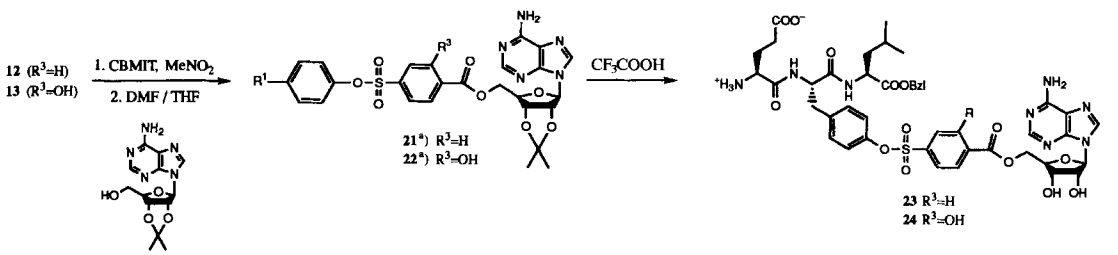
Scheme 2



[a]) For R^1, see *Scheme 1*. [b]) For R^2, see *Scheme 1*. DIEA = $(^iPr)_2EtN$, TBDMS = $^tBuMe_2Si$.

The protected complete transition-state analogs **21** and **22** (*Scheme 3*) were synthesized by reaction of the peptide-triphosphate analogs **12** and **13** with 2′,3′-*O*-isopropylideneadenosine (**20**) using 1,1′-carbonylbis(imidazolium) triflate (CBMIT) [34] as condensating agent. Removal of the protecting groups with CF_3COOH then gave the free compounds **23** and **24**, respectively.

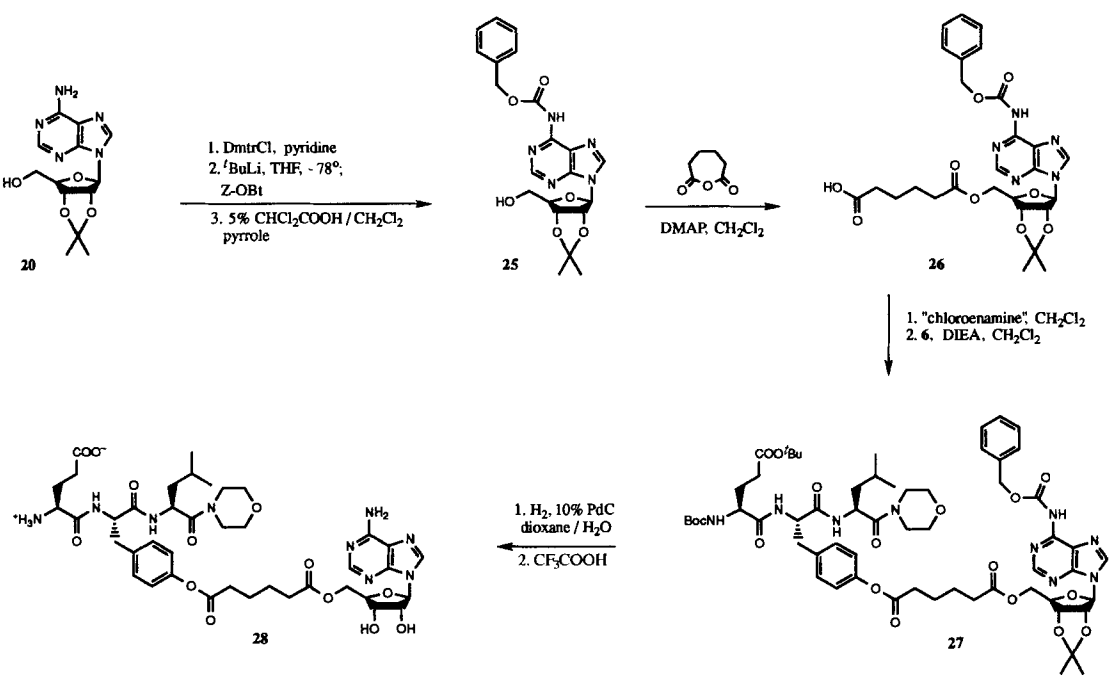
Scheme 3



ᵃ) For R^1, see *Scheme 1*.

For the preparation of the protected 5′-adenosyl ester **27** (*Scheme 4*), a different synthetic strategy was applied: the ATP analog **26** was attached to the tripeptide **6**. Thus, the N^6-Z-protected derivative **25**, which was obtained in a three-step reaction from **20**, was reacted with adipic anhydride [35] in the presence of 4-(dimethylamino)pyridine (DMAP) to give **26**, which then was converted to the corresponding acyl chloride by the

Scheme 4



DMAP = 4-(dimethylamino)pyridine, DIEA = $(^iPr)_2EtN$

mild 1-chloro-*N*,*N*,2-trimethylprop-1-en-1-amine ('chloroenamine') method [36] [37] and subsequently treated *in situ* with **6** to yield **27**. The Boc ^{t}Bu, and isopropropylidene groups of **27** were removed using first catalytic hydrogenation over Pd/C and then CF_3COOH to yield the free compound **28**. All compounds synthesized were characterized by mass and ^{1}H- and ^{13}C-NMR spectra (see *Exper. Part* for selected data).

Results and Discussion. – In our earlier work on EGF-R protein tyrosine kinase inhibitors [18–20], a β-nitrostyrene derivative was used as the tyrosine mimic. Assuming that this nitrostyrene part would indeed interact with the tyrosine binding site of the target enzyme, even better inhibitors might come from a replacement of the nitrostyrene with a short tyrosine-containing peptide. However, it has to be born in mind that tyrosine itself or a tyrosine-containing peptide might interact in a completely different way with the enzyme than the *Michael* acceptor nitrostyrene.

The compounds we synthesized were tested as EGF-R tyrosine kinase inhibitors using the purified recombinant intracellular domain of EGF-R (EGF-R ICD) and angiotensin II as the P-accepting substrate (*Table 1*). The results presented here indicate that our combinations of building blocks only led to moderately active inhibitors of the EGF-R tyrosine kinase. The best bisubstrate analog inhibitor in this study is **24**, containing the tripeptide H-Glu-Tyr-Leu-OBzl, the 2-hydroxy-4-sulfonylbenzoyl moiety, and adenosine (IC_{50} = 33 μM). The related compound **17**, lacking the adenosine, was also found to have a modest inhibitory activity (IC_{50} = 92 μM). The fact that **24** is about three times as active as **17** suggests that the adenosine part indeed adds to the inhibitory activity of the compound.

Table 1. *Inhibitory Activities of Compounds* **9**, **16**–**19**, **23**, **24**, **28**, *and* **29** *against EGF-RICD*[a])

	9	**16**	**17**	**18**	**19**	**23**	**24**	**28**	**29**
IC_{50} [μM][b])	inact.	inact.	92	inact.	inact.	inact.	33	inact.	68[c]) [38]

[a]) In addition, the protected intermediates **5**, **6**, **12**–**15**, **21**, **22**, and **27** were also tested routinely. With the exception of **13** (IC_{50} = 29 μM), they were inactive.

[b]) Inactive: no inhibitory effect observable at 100 μM.

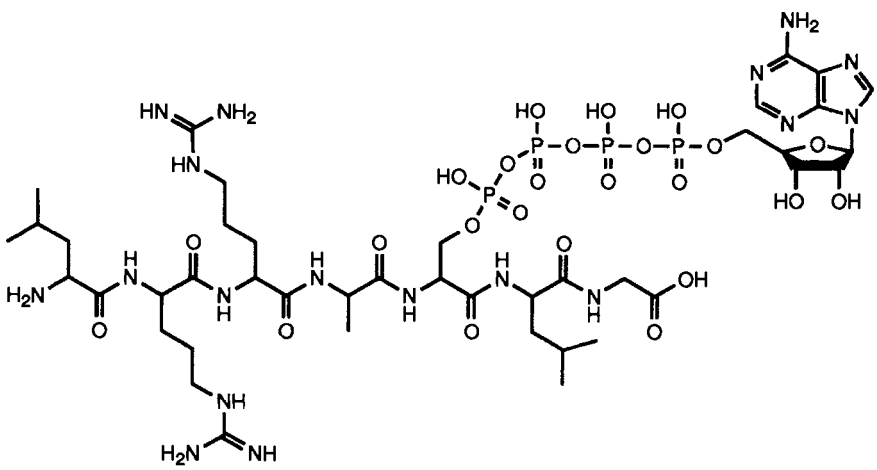
[c]) Determined with cAMP-dependent protein kinase.

The nature of the spacer part also seems to be of importance. A dramatic decrease in the activity against the EGF-R ICD was observed when the 4-sulfonylbenzoyl group was used as triphosphate substitute instead of the 2-hydroxy-4-sulfonylbenzoyl moiety (**23** *vs.* **24**) as was observed earlier for a different series of compounds [18]. The additional OH group may enhance the complexation of bivalent metal ions. Subtle changes in the length of the spacer unit or in the way it is attached to the tyrosine mimic also greatly influence the inhibitory activity as was shown in our earlier reports [19] [20]. In this respect, the adipoyl moiety of **28** is quite a long and flexible spacer, which might be one of the reasons why **28** proved to be inactive.

Elongation of the peptide moiety at the C-terminus with arginine and combination of the resulting tetrapeptide Glu-Tyr-Leu-Arg with the 2-hydroxy-4-sulfonylbenzoyl spacer (*cf.* compound **18**) or with a benzene-1,4-disulfonyl moiety (compound **19**) also

resulted in inactive compounds. In addition, our experiments established that the phosphotetrapeptide **9** has no inhibitory activity.

The tyrosine-containing peptide moieties that we used in the syntheses of the potential inhibitors described were in addition tested as substrates of the EGF-R tyrosine kinase (data not shown). This enzyme was not able to phosphorylate the tyrosine moiety, probably due to rather weak interactions between the enzyme and these peptides. Nevertheless, the combination **24** of one of these peptide moieties with a spacer and an adenosine derivative led to an inhibitory activity which was in the same range than that reported recently by *Gibson* and coworkers for the adenosine 5′-tetraphosphate heptapeptide **29** (IC_{50} = 68 μM with cAMP-dependent protein kinase) [38]. It is noteworthy that the peptide moiety of **29** was based on the well-known substrate peptide of the cAMP-dependent protein kinase.



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A possible explanation for the low activity of our compounds might, indeed, be the rather weak or unspecific interactions between the enzyme and the peptide moieties. Also, the influence of polar, charged end groups at the N-terminus, or of the nonpolar morpholides or benzyl esters at the C-terminus of our compounds are hardly predictable in this respect. Optimized peptide sequences with higher affinity to the EGF-R PTK will probably be required if the synthesis of bisubstrate analogs of this type is to lead to compounds showing significant inhibitory activity.

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Experimental Part

General. All chemicals were purchased from *Fluka AG, Aldrich, Bachem, Novabiochem*, or *Ciba-Geigy AG* in *purum* or *puriss. p.a.* quality. Solvents used in reactions were distilled and dried or purchased in *abs.* quality. THF was freshly distilled from Na/K. DMF was passed through a column filled with glass wool, neutral, basic, and

acidic Al_2O_3. Glassware was dried with a flame and cooled under Ar. Org. extracts were dried (Na_2SO_4), evaporated on a rotary evaporater (< 35°), and dried under high vacuum. TLC: *Merck* silica gel *60* F_{254} precoated glass plates. Prep. TLC: *Merck* silica gel *60* $F_{254+366}$ precoated 'PSC' glass plates (2 mm). Flash chromatography (FC): procedure of *Still et al.* [39] with H_2O-cooled columns; *Merck* silica gel *60*, 40–63 μm. Anal. HPLC (purity control): *Merck Hitachi* gradient HPLC anal. system, *VYDAC* diphenyl reversed-phase column (5 μm, 4.6 × 150 mm; *Paul Bucher*, Basel); flow rate 1 ml/min; solvents 0.1% CF_3COOH in MeCN (*A*), 0.1% CF_3COOH in H_2O (*B*); gradient: within 30 min from 5% *B* to 100% *B*; detection at 214 nm; t_R in min. Prep. MPLC: *Büchi* MPLC system with *Knauer* variable-wavelength UV monitor; *Merck Lichroprep RP-18*, 15–25 μm; flow rate 60 ml/min; solvents: 0.1% CF_3COOH in H_2O (*A*), 0.1% CF_3COOH in MeCN (*B*). Optical rotation: *Perkin-Elmer* polarimeter model *141*. M.p.: *Kofler* hot stage; uncorrected. NMR: *Varian VXR-400* (^1H, 400 MHz; ^{13}C, 101 MHz) or *Varian Gemini 300* (^1H, 300 MHz; ^{13}C, 75 MHz; ^{31}P, 121 MHz); δ in ppm rel. to internal Me_4Si and external 85% aq. H_3PO_4 soln. resp. (= 0 ppm), coupling constants *J* in Hz; multiplicities of ^{13}C resonances from APT and H,C-COSY experiments; * means that assignments may be interchanged. FAB-MS: *VG 70-250* or *ZAB-HF*; matrix: 3-nitrobenzyl alcohol (NBA). ^{252}Cf-PD-MS: *BIO-ION-20* plasma desorption instrument; samples were applied to a nitrocellulose matrix. MALDI-MS: *LDI-1700* mass monitor; matrices: 2,5-dihydroxybenzoic acid (2,5-DHB), 2,6-dihydroxyacetophenone (2,6-DHA), diammonium citrate (DAHC), α-cyano-4-hydroxycinnamic acid (α-CHC).

Biological Materials: Angiotensin II was purchased from *Sigma Chemicals Ltd.*, St Louis, USA, or from *Fluka AG*. [γ-^{32}P]ATP was from *Amersham Corp*. The intracellular domain of the EGF-R (EGF-R ICD) was expressed in Sf9 cells using recombinant baculoviruses and purified as previously described [40].

Boc-Glu(O^{t}Bu)-Tyr-Leu-OBzl (**5**). To a soln. of HCl · H-Tyr-Leu-OBzl (3.46 g, 8.22 mmol) and $(^iPr)_2EtN$ (1.50 ml, 8.22 mmol) in DMF (80 ml), a soln. of Boc-Glu(O^{t}Bu)-OSu (3.31 g, 8.22 mmol) in DMF (50 ml) was added at 5°. After stirring for 23 h at r.t., the solvent was evaporated, the resulting oil dissolved in AcOEt (100 ml), the soln. washed with H_2O, 10% aq. citric acid soln., sat. aq. Na_2CO_3 soln., and brine, dried, and evaporated. FC (CH_2Cl_2/MeOH 98:2, 97:3, 95:5) gave **5**, (5.19 g, 94%). Colorless amorphous powder. M.p. 61–63°. $[\alpha]_D^{24} = -21.2$, $[\alpha]_{365}^{24} = -67.4$ ($c = 2.03$, $CHCl_3$). FAB-MS (NBA): 670(15, $[M + H]^+$), 514(3, $[M - Boc - {}^tBu]^+$), 337(5), 265(3), 247(3), 222(18), 191(5), 147(13, Glu^+), 136(40), 120(3), 102(20), 91(100, $PhCH_2^+$), 77(10, Ph^+), 57(88, $^tBu^+$).

Boc-Glu(O^{t}Bu)-Tyr-Leu-morpholine (**6**). A soln. of **5** (3.67 g, 5.48 mmol) in EtOH/H_2O 9:1 (150 ml) was hydrogenated at r.t. (H_2, 1 atm) over 10% Pd/C (1.83 g). After 4 h, the catalyst was filtered off and washed with EtOH/H_2O 9:1, the soln. evaporated, and the resulting solid dried under high vacuum: pure Boc-Glu(O^{t}Bu)-Tyr-Leu-OH (3.16 g, 99.5%). M.p. 95–100°. $[\alpha]_D^{22} = -14.5$, $[\alpha]_{365}^{22} = -47.9$, ($c = 2.03$, $CHCl_3$). FAB-MS (NBA/KCl): 618(25, $[M + K]^+$), 580(6, $[M + H]^+$), 486(6, $[M - Leu]^+$), 337(5), 265(4), 221(3), 191(3), 147(6, Glu^+), 136(36), 120(3), 102(20), 86(18), 77(20), 57(100, tBu^+).

To a soln. of Boc-Glu(O^{t}Bu)-Tyr-Leu-OH (522 mg, 900 μmol) und freshly distilled *N*-methylmorpholine (100 μl, 900 μmol) in abs. CH_2Cl_2 (10 ml), isobutyl chloroformate (120 ml, 918 μmol; freshly distilled under Ar) was added at −15°. After 5 min, freshly distilled morpholine (100 μl, 1.15 μmol) was slowly added at −15°. The mixture was stirred for 25 min at 0° and for 2.5 h at r.t., washed with H_2O, 10% aq. citric acid soln., sat. aq. $NaHCO_3$ soln., and brine, dried, and evaporated. FC (CH_2Cl_2/MeOH 95:5, 9:1) afforded **6** (521 mg, 89%). Colorless amorphous powder. M.p. 97–101°. $[\alpha]_D^{25} = -18.1$, $[\alpha]_{365}^{25} = -64.0$, ($c = 2.13$, $CHCl_3$). FAB-MS (NBA): 649(21, $[M + H]^+$), 562(3, $[M - morpholine]^+$), 506(3, $[M - morpholine - {}^tBu]^+$), 450(8), 337(5), 309(3), 293(3), 265(3), 247(3), 232(5), 201(10), 152(3), 136(47), 102(22), 86(86, $[morpholine]^+$), 77(12, Ph^+), 57(100, $^tBu^+$).

(tert-*Butyl)dimethylsilyl 4-(Chlorosulfonyl)benzoate* (**10a**). To a soln. of 4-(chlorosulfonyl)benzoic acid (364 mg, 165 mmol) in dry THF (3 ml), $CF_3CON(Me)Si(^tBu)Me_2$ containing 1% of $(^tBu)Me_2SiCl$ (767 μl, 3.30 mmol) was added under Ar. The orange mixture was stirred for 20 min and evaporated at 35° under high vacuum. Orange, amorphous solid. M.p. 105–108°.

Boc-Glu(O^{t}Bu)-Tyr[$SO_2C_6H_4$(4-COOH)]-Leu-OBzl (**12**). To a suspension of **5** (790 mg, 1.30 mmol) and molecular sieves (powder 4 Å) in abs. CH_2Cl_2 (6 ml) under Ar, $(^iPr)_2Et_2N$ (225 μl, 1.33 mmol) and a soln. of **10a** (474 mg, 1.42 mmol) in abs. CH_2Cl_2 (2 ml) were slowly added at −3°. After stirring for 26 h at r.t. and addition of CH_2Cl_2, the mixture was filtered, washed with H_2O, dried, and evaporated. FC (CH_2Cl_2/MeOH 98:2, 95:5, 90:10) yielded pure **12** (451 mg, 45%) and 499 mg (43%) of silyl-protected derivative of **12** as a light-yellow solid.

12: M.p. 125–129°. $[\alpha]_D^{25} = -28.8$, $[\alpha]_{365}^{25} = -103.9$ ($c = 1.41$, $CHCl_3$). ^1H-NMR (300 MHz, $(CD_3)_2SO$): *Table 2*. ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): *Table 3*. FAB-MS (NBA): 854(5, $[M + H]^+$), 742(4), 320(12), 222(7), 202(3), 147(5, Glu^+), 102(20), 91(100, $PhCH_2^+$), 77, (10, Ph^+), 57(88, $^tBu^+$).

Silyl-protected derivative of **12**: M.p. 53–56°. $[\alpha]_D^{25} = -18.1$, $[\alpha]_{365}^{25} = -64.0$ ($c = 2.53$, $CHCl_3$). FAB-MS (NBA): 968(1, $[M + H]^+$), 434(9), 222(8), 178(6), 136(6), 91(100, $PhCH_2^+$), 73(22), 57(68, $^tBu^+$).

Table 2. *^{1}H-NMR Data of Compounds* **12**, **13**, **16**–**18**, **23**, **24**, *and* **28**[a])

	12	**13**	**16**	**17**	**18**	**23**	**24**	**28**
Glu:								
H–C(2)[b])	3.74–3.87 (*m*)	3.84–3.88 (*m*)	3.76 (br. *s*)	3.74–3.76 (*m*)	4.13–4.18 (*m*)	[d])	[d])	3.74–3.77 (*m*)
CH_2(3)[c])	1.52–1.69 (*m*)	1.51–1.71 (*m*)	1.91–1.94 (*m*)	1.91–1.96 (*m*)	1.43–1.81 (*m*)	1.92–2.10 (*m*)	1.91–1.93 (*m*)	1.93–1.96 (*m*)
CH_2(4)	2.10 (*t*, *J* = 7.5)	2.07–2.10 (*m*)	2.29–2.34 (*m*)	2.28–2.34 (*m*)	2.15 (*t*, *J* = 7.8)	2.31–2.37 (*m*)	2.29–2.33 (*m*)	2.34–2.39 (*m*)
Boc, ^{t}Bu, Ac	1.36, 1.38 (2*s*)	1.36, 1.39 (2*s*)			1.81 (*s*)			
Tyr:								
H–C(2)[b])	4.32–4.38 (*m*)	4.33–4.36 (*m*)	4.34–4.38 (*m*)	4.32–4.40 (*m*)	4.13–4.18 (*m*)	4.28–4.71 (*m*)	4.35–4.70 (*m*)	4.60–4.65 (*m*)
CH_2(3)	2.72 (*dd*, *J* = 9.5, 13.5); 2.91 (*dd*, *J* = 2.5, 13.5)	2.71 (*dd*, *J* = 9.5, 14); 2.91 (*dd*, *J* = 3, 14)	2.73 (*dd*, *J* = 9.5, 14.5); 2.94 (*dd*, *J* = 4, 14.5)	2.72 (*dd*, *J* = 10, 14); 2.94 (*dd*, *J* = 3.5, 14)	2.72 (*dd*, *J* = 9.5, 13.5); 3.03 (*dd*, *J* = 3.5, 14)	2.74 (*dd*, *J* = 10, 14); 2.95 (*dd*, *J* = 4, 14)	2.75 (*dd*, *J* = 9, 13); 2.95 (*dd*, *J* = 3, 13.5)	2.79 (*dd*, *J* = 10, 13.5); 3.02 (*dd*, *J* = 3, 14)
H–C(2′,6′)	7.19 (*d*, *J* = 8)	7.18 (*d*, *J* = 8.5)	7.26 (*d*, *J* = 8.5)	7.26 (*d*, *J* = 8.5)	7.14 (*d*, *J* = 8.5)	7.26 (*d*, *J* = 8.5)	7.26 (*d*, *J* = 8.5)	7.33 (*d*, *J* = 8.5)
H–C(3′,5′)	6.85 (*d*, *J* = 8)	6.86 (*d*, *J* = 8.5)	6.95 (*d*, *J* = 8.5)	6.96 (*d*, *J* = 8.5)	6.86 (*d*, *J* = 8.5)	6.96 (*d*, *J* = 8.5)	6.97 (*d*, *J* = 8.5)	7.03 (*d*, *J* = 8)
Leu:								
H–C(2)[b])	4.51–4.55 (*m*)	4.52–4.58 (*m*)	4.55–4.58 (*m*)	4.51–4.56 (*m*)	4.24–4.27 (*m*)	4.28–4.71 (*m*)	4.35–4.70 (*m*)	4.72–4.77 (*m*)
CH_2(3), H–C(4)[c])	1.52–1.69 (*m*)	1.51–1.71 (*m*)	1.52–1.61 (*m*)	1.52–1.61 (*m*)	1.44–1.77 (*m*)	1.54–1.58 (*m*)	1.54–1.61 (*m*)	1.58–1.62 (*m*)
2Me-C(4)	0.83, 0.89 (2*d*, each *J* = 6)	0.83, 0.89 (2*d*, each *J* = 6)	0.81, 0.88 (2*d*, each *J* = 6.5)	0.82, 0.88 (2*d*, each *J* = 6)	0.82, 0.88 (2*d*, each *J* = 6.5)	0.82, 0.89 (2*d*, each *J* = 6)	0.81, 0.88 (2*d*, each *J* = 6)	0.86, 0.89 (2*d*, each *J* = 6.5)
$PhCH_2$	5.12 (*s*)	5.13 (*s*)	5.10 (*s*)	5.11 (*s*)		5.10 (*s*)	5.10 (*s*)	[e])
PhCH_2	7.31–7.36 (*m*)	7.32–7.36 (*m*)	7.29–7.37 (*m*)	7.30–7.35 (*m*)		7.29–7.35 (*m*)	7.29–7.34 (*m*)	
Arg:								
					4.44–4.48 (*m*)			
					1.44–1.77 (*m*)			
					3.10 (*q*, *J* = 6)			
Amide resonances:								
NH	6.88 (*d*, *J* = 9); 7.90 (*d*, *J* = 8); 8.51 (*d*, *J* = 7.5)	6.86[d]); 7.81 (*d*, *J* = 8.5); 8.48 (*d*, *J* = 7.5)	8.12 (br. *s*); 8.60 (*d*, *J* = 8); 8.71 (*d*, *J* = 8)	8.10 (br. *s*); 8.59 (*d*, *J* = 8); 8.67 (*d*, *J* = 9)	7.64 (*t*, *J* = 5.5); 7.82 (*d*, *J* = 8); 8.00–8.03 (*m*, 3H)	8.10 (br. *s*); 8.60 (*d*, *J* = 6); 8.71 (*d*, *J* = 8)	8.05 (br. *s*); 8.60 (*d*, *J* = 7.5); 8.71 (*d*, *J* = 7.5)	8.51 (*d*, *J* = 8); 8.66 (*d*, *J* = 8)
NH_2					7.09 (*s*, 1H); 7.31 (*s*, 1H)			

Table 2 (cont.)

	12	**13**	**16**	**17**	**18**	**23**	**24**	**28**
Spacer:								
H–C(2), H–C(6)	7.81 (*d*, *J* = 8.5)	6.97–7.00 (*m*)	7.99 (*d*, *J* = 8.5)	7.18–7.21 (*m*)	7.03–7.05 (*m*)	8.02 (*d*, *J* = 8.5)	7.38 (*d*, *J* = 10)	2.34–2.39 (*m*)[f])
H–C(3), H–C(5)	8.14 (*d*, *J* = 8.5)	7.87 (*d*, *J* = 8.5)	8.18 (*d*, *J* = 8.5)	7.87 (*d*, *J* = 8)	7.89 (*d*, *J* = 8.5)	8.20 (*d*, *J* = 8.5)	7.93 (*d*, *J* = 8.5)	1.58–1.62 (*m*)[g])
CH_2(5)								2.52–2.57[d])
Adenosine:								
H–C(1′)						6.01 (*d*, *J* = 4.5)	5.96 (*d*, *J* = 5)	5.92 (*d*, *J* = 5)
H–C(2′)						4.28–4.71 (*m*)	4.35–4.70 (*m*)	4.68 (*t*, *J* = 5)
H–C(3′)								4.27 (*t*, *J* = 5)
H–C(4′)								4.09–4.11 (*m*)
CH_2(5′)								4.21 (*dd*, *J* = 6, 12); 4.36 (*dd*, *J* = 3.5, 12)
H–C(2)						8.60 (*s*)	8.42 (*s*)	8.16 (*s*)
H–C(8)						8.30 (*s*)	8.19 (*s*)	8.32 (*s*)
NH_2						[d])	[d])	7.3 (*s*)

[a]) Chemical shifts in ppm rel. to internal $SiMe_4$ measured in $(CD_3)_2SO$; coupling constants *J* in Hz; OH resonances not reported.
[b]) Assignments within the same column may be interchanged.
[c]) Assignments within the same column may be interchanged.
[d]) Resonance observed with difficulty or not observed due to broadening or overlap.
[e]) Morpholine resonances not observed due to overlap.
[f]) CH_2(2).
[g]) CH_2(3), CH_2(4).

Table 3. *^{13}C-NMR Data of Compounds* **12, 13, 16–18, 23, 24,** *and* **28**[a])

	12	**13**	**16**	**17**	**18**	**23**	**24**	**28**
Glu:								
C(1)[b])	170.8	170.8	168.2	168.3	169.8	168.3	168.2	168.5
C(2)[b])	53.5	53.6	53.6	53.7	51.8	53.7	53.7	54.1
C(3)	27.2	27.2	26.4	26.4	27.0	26.5	26.4	26.5
C(4)	31.1	31.2	28.8	28.7	30.2	28.8	28.7	28.9
C(5)[b])	171.5	171.8	173.4	173.4	173.1	173.4	171.5	173.5
Boc, ^{t}Bu, Ac	27.6, 28.0	27.7, 28.0			22.4			
Boc, ^{t}Bu, Ac	78.1, 79.5	78.2, 79.5			170.7			
Boc	155.0	155.1						
Tyr:								
C(1)[b])	171.1	171.6	170.7	170.7	171.4	170.7	171.1	170.2
C(2)[b])	52.7	52.5	51.3	51.2	52.0	51.3	52.7	51.4
C(3)	36.9	36.9	38.7	36.2	36.1	36.4	36.1	36.5
C(1')[b])	135.6	135.8	135.8	135.8	137.2[b])	135.8	135.8	135.0
C(2',6')	128.3	130.5[b])	128.6	130.5	130.5	128.7	130.7	130.1
C(3',5')	121.3	121.3	121.6	121.6	121.5	121.7	121.6	121.4
C(4')	147.4	147.6	147.4	147.6	147.6	147.4	147.4	149.4[b])
Leu:								
C(1)[b])	171.9	171.9	171.9	171.9	171.7	172.0	171.9	171.7
C(2)[b])	50.2	50.3	50.4	50.3	53.4	50.4	50.3	46.5
C(3)	40.0	–	40.1	–	40.5	–	40.3	40.6
C(4)	24.0	24.1	24.2	24.2	24.1	24.2	24.1	24.1
2*Me*–C(4)	21.1, 22.6	21.2, 22.6	21.1, 22.7	21.1, 22.7	21.4, 23.1	21.1, 22.7	21.0, 22.8	21.5, 23.1
$PhCH_2$	65.8	66.0	66.0	66.0		66.0	66.0	41.9, 45.5[c])
PhCH_2(*o*, *m*, *p*)	127.7, 127.9, 128.3	127.8, 128.0, 128.3	127.8, 128.0, 128.4	127.8, 128.0, 128.3		127.8, 128.1, 128.4	127.8, 128.0, 128.3	66.3[c])
PhCH_2(*ipso*)	136.8	136.7	137.0	136.9		137.1	137.0	
Arg:								
					173.9			
					52.1			
					29.2			
					24.9			
					40.4			
					156.7			

Table 3 (cont.)

	12	**13**	**16**	**17**	**18**	**23**	**24**	**28**
Spacer:								
C(1)	135.8[b])	136.6[b])	137.9[b])	138.1	137.0[b])	138.4[b])	138.9[b])	172.6[b])
C(2)	130.7[b])	114.3[b])	130.6[b])	116.2	115.1[b])	130.7[b])	116.6[b])	33.9[b])
C(3)	130.0[b])	164.1	130.6[b])	162.0	163.4	130.5[b])	158.4	23.7
C(4)	135.8[b])	124.8	136.3[b])	122.0	124.2	134.8[b])	119.1	23.7
C(5)	130.0[b])	130.8[b])	130.6[b])	131.5	115.7[b])	130.5[b])	132.1	33.1[b])
C(6)	130.7[b])	115.6[b])	130.6[b])	116.2	131.1	130.7[b])	117.8[b])	169.9[b])
C=O	167.4	169.4	165.8	169.6	169.6	164.2	165.6	
Adenosine:								
C(1')						88.2	88.2	87.8
C(2')						73.2	73.2	72.8
C(3')						70.2	70.2	70.3
C(4')						81.6	81.6	81.5
C(5')						65.5	65.5	63.8
C(2)						147.8	147.8	152.6
C(4)						148.6	148.6	149.1[b])
C(5)						119.0	119.0	119.1
C(6)						158.7	158.7	156.0
C(8)						141.7	141.7	139.7

[a]) Chemical shifts in ppm rel. to internal $SiMe_4$ measured in $(CD_3)_2SO$.
[b]) Assignments of similar chemical shifts within the same column may be interchanged.
[c]) Morpholine resonances.

H-Glu-Tyr[$SO_2C_6H_4$(4-COOH)]-Leu-OBzl (**16**). To **12** (80.0 mg, 93.6 μmol), CF_3COOH (400 μl) was added. The orange mixture was stirred for 20 min at r.t. under Ar. Precipitation of the product by adding $(^iPr)_2O$, filtration, and lyophilization of the precipitate from H_2O afforded **16** (68.8 mg, *ca.* quant.). Colorless, amorphous powder. M.p. 131–136°. $[\alpha]_D^{26} = +0.8$, $[\alpha]_{365}^{26} = +23.2$ ($c = 1.11$, MeOH). ^{1}H-NMR (400 MHz, $(CD_3)_2SO$): *Table 2.* ^{13}C-NMR (101 MHz, $(CD_3)_2SO$): *Table 3.* FAB-MS (thioglycerol): 736(3, $[M + K]^+$), 720 (6, $[M + Na]^+$), 698(32, $[M + H]^+$), 449(4), 369(7), 320(12), 265(8), 222(17), 192(11), 147(10), 102(23).

(tert-*Butyl*)*dimethylsilyl 2-[*(tert-*Butyl*)*dimethylsilyloxy]-4-*(*chlorosulfonyl*)*benzoate* (**10b**). As described for **10a**, with 4-(chlorosulfonyl)-2-hydroxybenzoic acid (178 mg, 750 μmol), dry THF (1 ml), and $CF_3CON(Me)Si(^tBu)Me_2$ containing 1% of $^tBuMe_2SiCl$ (700 μl, 3.00 mmol). The mixture was stirred for 40 min. Evaporation (35°, high vacuum) afforded **10b** as a dark orange oil.

Boc-Glu(O^{t}Bu)-Tyr[$SO_2C_6H_3$(3-OH)(4-COOH)]-Leu-OBzl (**13**). As described for **12**, with **5** (268 mg, 400 μmol), molecular sieves (powder 4 Å), abs. CH_2Cl_2 (3 ml, $(^iPr)_2EtN$ (75 μl, 400 μmol), **10b** (230 mg, 480 μmol), and abs. CH_2Cl_2 (3 ml) for 5 min at −5°, then 20 h at r.t. FC (CH_2Cl_2/MeOH 98:2, 97:3, 90:10) gave pure **13** (31.6 mg, 9%) as a light yellow solid and 245 mg (56%) of the silyl-protected derivative of **13**. **13**: M.p. 150–155°. $[\alpha]_D^{26} = -48.0$, $[\alpha]_{365}^{26} = -97.7$ ($c = 2.13$, $CHCl_3$). ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): *Table 2.* ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): *Table 3.* FAB-MS (thioglycerol): 892(2, $[M + Na]^+$), 870(1, $[M + H]^+$), 814(1, $[M - {}^tBu]^+$), 770(2, $[M - Boc]^+$), 714(5), 585(3), 336(30), 222(25), 202(3), 147(23, Glu^+), 136(22), 102(21).

H-Glu-Tyr[$SO_2C_6H_3$(3-OH)(4-COOH)]-Leu-OBzl (**17**). As described for **16**, with **13** (67.4 mg, 77.5 μmol and CF_3COOH (400 μl). Usual workup gave **17** (51.3 mg, *ca.* quant.). Colorless powder. M.p. 155–162°. $[\alpha]_D^{26} = +4.7$, $[\alpha]_{365}^{26} = -14.6$ ($c = 1.07$, MeOH). ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): *Table 2.* ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): *Table 3.* FAB-MS (thioglycerol): 752(12, $[M + K]^+$), 714(4, $[M + H]^+$), 465(2), 336(8), 265(6), 222(10), 147(12, Glu^+), 102(17).

Boc-Glu(O^{t}Bu)-Tyr{$SO_2C_6H_4$[4-[CO-A(>CMe_2)]]}-Leu-OBlz (**21**). To a suspension of **12** (342 mg, 400 μmol), 1-methyl-1*H*-imidazol (3.2 μl, 40 μmol), and molecular sieves (powder 4 Å) in abs. $MeNO_2$ (1 ml) under Ar, a soln. of CBMIT in $MeNO_2$ (0.8M, 1 ml; prepared according to [34]) was slowly added. After 2 h, a soln. of 2′,3′-*O*-isopropylideneadenosine (**20**; 99.0 mg, 320 μmol) in THF/DMF 6:1 (3.5 ml) was added. The dark green mixture was stirred for 17 h and evaporated, the residue dissolved in CH_2Cl_2 (15 ml), and the soln. washed with H_2O and brine, dried, and evaporated. FC (CH_2Cl_2/MeOH 98:2, 97:3, 95:5, 9:3) afforded **21** (243 mg, 53%). Colorless oil with minor impurities. FAB-MS (thioglycerol): 1143(8, $[M + H]^+$), 1043 (2, $[M - Boc]^+$), 987(7, $[M - Boc - {}^tBu]^+$), 411(10), 222(20), 136(85, [adenine + $H]^+$).

H-Glu-Tyr{$SO_2C_6H_4$[4-(CO-A)]}-Leu-OBzl (**23**). As described for **16**, with **21** (61.0 mg, 53.5 μmol) and CF_3COOH (350 μl; 1 h). Usual workup gave **23** (53.0 mg, *ca.* quant.) Colorless powder (NMR: small amount of isopropylidene protecting group still present). ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): *Table 2.* ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): *Table 3.* FAB-MS (thioglycerol): 947(25, $[M + H]^+$), 698(10), 514(30), 217(28), 136(45, [adenine + $H]^+$), 102(20).

Boc-Glu(O^{t}Bu)-Tyr{$SO_2C_6H_3$(3-OH){4-[CO-A(>CMe_2)]}}-Leu-OBzl (**22**). As described for **21**, with **13** (254 mg, 292 μmol), 1-methyl-1*H*-imidazol (2.4 μl, 30.0 μmol), molecular sieves (powder 4 Å), abs. $MeNO_2$ (2 ml), CBMIT soln. (1 ml, 730 μmol), 2′,3′-*O*-isopropylideneadenosine (**20**; 72 mg, 234 μmol), and THF/DMF 4:3 (1.75 ml). The mixture was stirred for 24 h. FC (CH_2Cl_2/MeOH 98:2, 97:3, 95:5, 9:2) yielded **22** (55.8 mg, 16%). Colorless oil with minor impurities. FAB-MS (NBA): 1159(100, $[M + H]^+$), 1003(14, $[M - {}^tBu]^+$), 427(38), 277(8), 222(38).

H-Glu-Tyr{$SO_2C_6H_3$(3-OH)[4-(CO-A)]}-Leu-OBzl (**24**). As described for **16**, with **22** (25.0 mg, 21.7 μmol) and CF_3COOH (200 μl). The mixture was stirred for 1 h. Usual workup gave **24** (22.8 mg, *ca.* quant.). Colorless powder (NMR: small amount of isopropylidene protecting group still present). ^{1}H-NMR (400 MHz, $(CD_3)_2SO$): *Table 2.* ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): *Table 3.* FAB-MS (NBA/KCl): 1003(1, $[M + K]^+$), 963(5, $[M + H]^+$), 136(31, [adenine + $H]^+$), (100, $PhCH_2^+$), 77(12, Ph^+).

N^6-[(*Benzyloxy*)*carbonyl*]-2′,3′-O-*isopropylideneadenosine* (**25**). A soln. of 2′,3′-*O*-isoproylideneadenosine (**20**; 1.41 g, 4.58 mmol) and DmtrCl (1.86 g, 5.49 mmol) in abs. pyridine (8 ml) was stirred for 5 h under Ar. The light orange mixture was evaporated, the residue dissolved twice in toluene and the soln. evaporated, and the residue dried. FC (CH_2Cl_2, CH_2Cl_2/MeOH 98:2, 96:4) afforded 5′-O-[(4,4′-*dimethoxytriphenyl*)*methyl*]-2′,3′-O-*isopropylideneadenosine* (1.11 g, 1.83 mmol, 40%). Yellowish powder with minor impurities. M.p. 90–94°. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): 8.27(*s*, H–C(2)); 8.01(*s*, H–C(8)); 7.30–7.32(*m*, arom. H); 7.13–7.27(*m*, arom. H); 6.79, 6.76(2*d*, $J = 9.0$, arom. H); 6.20(*d*, $J = 2.0$, H–C(1′)); 5.45(*dd*, $J = 6.3$, 1.9, H–C(2′)); 4.97(*dd*, $J = 6.0$, 3.2, H–C(3′)); 4.27–4.30(*m*, H–C(4′)); 3.72, 3.71(2*s*, 2 MeO); 3.19(*dd*, $J = 9.7$, 6.4, 1H–C(5′)); 3.10(*dd*, $J = 10.0$, 4.6, 1H–C(5′)); 1.53, 1.31(2*s*, Me_2C). FAB-MS (NBA): 610(18, $[M + H]^+$), 303(100, $[M - Dmtr]^+$), 136(12, [adenine + $H]^+$), 77(6, Pl_2^+).

At 50°, 5′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-2′,3′-*O*-isopropylideneadenosine (614 mg, 1.00 mmol) was dried for 4 h under high vacuum, dissolved in abs. THF (5 ml) under Ar, and then cooled to −78°. 'BuLi in pentane (1.5M; 1.35 ml, 2.02 mmol) was slowly added. After 10 min at −78°, a soln. of benzyl 1*H*-benzotriazol-1-yl carbonate (Z-OBt; 383 mg, 1.51 mmol) in dry THF (2.5 ml) was added. The yellow mixture was stirred for 5 min at −78° and 15 min at r.t., quenched with H_2O (1 ml), evaporated, and lyophilized. FC (CH_2Cl_2, CH_2Cl_2/MeCN 9:1, 4:1, 3:1) gave pure N^6-*[(benzyloxy)carbonyl]-5′-O-[(4,4′dimethoxytriphenyl)methyl]-2′,3′-O-isopropylideneadenosine* (396 mg, 53%). Colorless crystals. M.p. 95–97°. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): 10.73 (br. *s*, NH); 8.56* (*s*, H–C(2)); 8.47* (*s*, H–C(8)); 7.09–7.48 (*m*, arom. H; 6.78, 6.74 (2*d*, *J* = 8.9, arom. H); 6.31 (*d*, *J* = 1.8, H–C(1′)); 5.48 (*dd*, *J* = 6.2, 1.8, H–C(2′)); 5.22 (*s*, PhCH_2); 4.98 (*dd*, *J* = 6.2, 3.2, H–C(3′)); 4.32–4.37 (*m*, H–C(4′)); 3.70, 3.69 (2*s*, 2 MeO); 3.19 (*dd*, *J* = 10.1, 6.9, 1H–C(5′)); 3.09 (*dd*, *J* = 10.2, 4.5, 1H–C(5′)); 1.54, 1.31 (2*s*, Me_2C). FAB-MS (NBA): 744 (11, $[M + H]^+$), 440 (5, $[M - Dmtr]^+$), 303 (100, $[M - Dmtr - Z]^+$), 135 (6, [adenine]$^+$), 91 (35, $PhCl_2^+$).

To N^6-[(benzyloxy)carbonyl]-5′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-2′,3′-*O*-isopropylideneadenosine (354 mg, 476 μmol) in CH_2Cl_2 (5.90 ml), 1*H*-pyrrole (294 μl) and 5% $CHCl_2COOH$ in CH_2Cl_2 (6 ml) were added. The orange-red mixture was stirred for 2 min at r.t. and quenched with sat. $NaHCO_3$ soln. (11.3 ml), the aq. phase extracted with CH_2Cl_2, and the combined org. phase dried and evaporated. Purification by FC (CH_2Cl_2, CH_2Cl_2/MeCN 9:1, 9:1.5, 9:2, 4:1, 1:1) yielded pure **25** (190 mg, 90%). Colorless crystals. M.p. 83–85°. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): 10.70 (br. *s*, NH); 8.66* (*s*, H–C(2)); 8.65* (*s*, H–C(8)); 7.34–7.48 (*m*, arom. H); 6.24 (*d*, *J* = 2.7, H–C(1′)); 5.41 (*dd*, *J* = 6.1, 2.7, H–C(2′)); 5.22 (*s*, $PhCH_2$); 5.13 (*t*, *J* = 5.3, OH); 4.99 (*dd*, *J* = 5.9, 2.5, H–C(3′)); 4.24–4.28 (*m*, H–C(4′)); 3.52–3.57 (*m*, CH_2(5′)); 1.56, 1.34 (2*s*, Me_2C). ^{13}C-NMR (101 MHz, $(CD_3)_2SO$): 152.0* (C=O); 151.7 (C(2)); 151.3* (C(6)); 149.7 (C(4)); 142.7 (C(8)); 136.3 (quat. C); 128.4, 127.9, 127.8 (arom. CH); 123.6 (C(5)); 113.0 (Me_3C); 89.8 (C(1′)); 86.8 (C(4′)); 83.5 (C(2′)); 81.3 (C(3′)); 66.2 (PhCH_2); 61.4 (CH_2(5′)); 27.0, 25.1 (Me_3C). FAB-MS (NBA): 442 (83, $[M + H]^+$), 270 (42, [adenosine]$^+$), 226 (23), 162 (15), 136 (17, [adenine + H]$^+$), 91 (100, $PhCH_2^+$), 59 (19).

N^6-[(*Benzyloxy)carbonyl]-2′,3′-*O*-*isopropylideneadenosine 5′-(Hydrogen Hexanedioate)* (**26**). To a soln. of **25** (179 mg, 405 μmol) in abs. CH_2Cl_2 (6 ml), adipic anhydride (78 mg, 608 μmol, prepared according to [35]) and 4-(dimethylamino)pyridine (0.2 mg) were added. The turbid mixture was stirred for 21 h and then evaporated, the resulting oil dissolved in AcOEt, and the soln. extracted with H_2O and 5 times with sat. $NaHCO_3$ soln. Acidification of the $NaHCO_3$ phase with 2N H_2SO_4, extraction with AcOEt, drying, and evaporation under high vacuum afforded **26** (148 mg, 64%). Colorless oil. FAB-MS (NBA): 570 (56, $[M + H]^+$), 508 (7), 270 (24, [adenosine]$^+$), 226 (34), 175 (15), 136 (20, [adenine + H]$^+$), 91 (100, $PhCH_2^+$), 77 (100, Ph^+), 55 (25).

Boc-Glu(O'Bu)-Tyr[CO(CH$_2$)$_4$CO-bzoc^{6}A(>CMe$_2$)]-Leu-morpholine (**27**). To a soln. of dry **26** (127 mg, 223 μmol) in abs. CH_2Cl_2 (5 ml), 1-chloro-*N*,*N*,2-trimethylprop-1-en-1-amine ('chloroenamine'; 40 μl, 267 μmol) [36] [37] and, after 15 min, $(^iPr)_2EtN$ (38 μl, 223 μmol) and **6** (87 mg, 134 μmol) in abs. CH_2Cl_2 (1 ml) were added under Ar at 0–5°. The mixture was stirred for 16 h at r.t. and for 5 h at 40° and then filtered and the soln. evaporated. The resulting oily, colorless residue (237 mg) was dissolved in AcOEt, the soln. washed with sat. $NaHCO_3$ soln. and H_2O, dried, and evaporated, and the resulting oil purified by prep. TLC (CH_2Cl_2/MeOH 95:5; extraction with CH_2Cl_2/MeOH 85:15): **27** (44.3 mg, 27%). Colorless, amorphous, sticky solid. HPLC: t_R 17.8. FAB-MS (NBA): 1200 (7, $[M + H]^+$), 270 (11), 226 (9), 201 (9), 136 (23, [adenine + H]$^+$), 86 (76, [morpholine]$^+$), 57 (100, $^tBu^+$).

H-Glu-Tyr(CO(CH$_2$)$_4$CO-A)-Leu-morpholine (**28**). A soln. of **27** (41.5 mg, 34.5 μmol) in dioxane/H_2O 7:3 (2.5 ml) was vigorously shaken under H_2 (1 atm, r.t.) in the presence of 10% Pd/C (4.5 mg). After 3 h, the catalyst was filtered off and washed with dioxane/H_2O 7:3. Lyophilization of the filtrate afforded Boc-Glu(O'Bu)-Tyr[CO(CH$_2$)$_4$CO-A(>CMe$_2$)]-Leu-morpholine, to which CF_3COOH (1 ml) was added. The orange mixture was stirred for 30 min at r.t. under Ar and poured into cold 'BuOMe. After stirring for 10 min, the precipitate was collected by centrifugation and the solvent removed by decantation. This procedure was repeated once and the precipitate then lyophilized from dioxane/H_2O to give pure **28** (21.1 mg, 65% rel. to **27**). Colorless, amorphous solid. HPLC: t_R 9. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): *Table 2*. ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): *Table 3*. FAB-MS (thioglycerol): 892 (7, $[M + Na]^+$), 870 (50, $[M + H]^+$), 621 (5), 378 (6), 201 (13), 164 (8), 136 (100, [adenine + H]$^+$).

Solid-Phase Peptide Synthesis: *Ac-Glu(O'Bu)-Tyr(Bzl)-Leu-Arg(Pmc)-OH* (**7**).

a) *Fmoc-Arg(Pmc)-HMPB-BHA-PS Resin*. In a vessel for manual synthesis, the HMPB-BHA-PS resin (10 g, 0.641 mmol/g) was washed 4 times with *N*,*N*-dimethylacetamide (DMA). Fmoc-Arg(Pmc)-OH (8.50 g, 6.41 mmol), abs. pyridine (2.60 ml, 32.0 mmol), 2,6-dichlorobenzoyl chloride (in 6 portions: 470 μl every hour, 19.2 mmol), and DMA (50 ml) were added successively, and the suspension was stirred for 14 h. The soln. was filtered, washed with DMA, 'PrOH (2×), MeOH (6×), and CH_2Cl_2 (2×), and dried under high vacuum. A sample of the resin (23.1 mg) was analyzed quantitatively for Fmoc content by treating it 4 times for 2 min with

20% piperidine in DMA (2 ml), twice with MeOH, and 4 times for 2 min with 20% piperidine in DMA (2 ml). The combined washings were diluted to 100 ml with MeOH, and the absorbance measured at 299.8 nm. The analysis showed a loading of 0.258 mmol/g. The resin was acetylated for 2 h with DMA/pyridine/Ac_2O 8:1:1 at r.t. and washed with DMA (3×).

b) *Ac-Glu(O^{t}Bu)-Tyr(Bzl)-Leu-Arg(Pmc)-HMPB-BHA-PS Resin.* The peptide was prepared on a semi-automatic shaking-vessel machine for manual addition of the activated Fmoc-amino acids. The following *General Procedure* was used for the stepwise addition of the Fmoc-protected amino acids Leu, Tyr, Glu to Fmoc-Arg (Pmc)-HMPB-BHA-PS resin (0.258 mmol/g): *1)* wash resin with ^{i}PrOH (2×0.5 min), *2)* wash with degassed DMA (2×0.5 min), *3)* wash with ^{i}PrOH (2×0.5 min), *4)* wash with degassed DMA (2×0.5 min), *5)* wash with 20% piperidine in DMA (1×0.2 min), *6)* wash with 20% piperidine in DMA (3×3 min), *7)* wash with degassed DMA (1×0.5 min), *8)* wash with ^{i}PrOH (1×0.6 min), *9)* wash with 20% piperidine in DMA (3×3 min), *10)* wash with degassed DMA (2×0.5 min), *11)* wash with ^{i}PrOH (2×0.4 min), *12)* wash with degassed DMA (3×0.5 min), *13)* wash with ^{i}PrOH (1×0.6 min), *14)* wash with degassed DMA (4×0.4 min) and quantitate the Fmoc cleavage from the absorption a 299.8 nm of the combined washings *5)–14)*, *15)* add preactivated Fmoc-amino acid (3 equiv. of the amino acid in 1-methyl-1*H*-pyrrolidone (NMP), 3 equiv. of 1.0N HOBt in NMP, and 3 equiv. of *N,N'*-diisopropylcarbodiimide (DICD) were stirred for 40 min prior to addition), shake resin for 1 h, remove 2 µl of the resin suspension for *Kaiser* test [41], *16)* wash with ^{i}PrOH (1×1 min), *17)* wash with degassed DMA (2×0.5 min), *18)* cap with DMA/pyridine/Ac_2O 8:1:1 (1×5 min), *19)* wash with degassed DMA (3×0.4 min). With Fmoc-Tyr (Bzl)-OH and Fmoc-Glu (O^{t}Bu)-OH, the *Kaiser* test was slightly positive after a 1-h coupling period, so these residues were recoupled using DICD/HOBt and TPTU, respectively (3 equiv. of the Fmoc-amino acid in NMP, 3 equiv. of 0.5N TPTU in NMP, and 3.3 equiv. of $(^i\text{Pr})_2$EtN in NMP were stirred 3 min prior to addition). After completion of chain assembly, the N-terminal Fmoc group was removed, the resin acetylated for 15 min with DMA pyridine Ac_2O 8:1:1, and the protected peptide-resin dried under high vacuum.

c) *Deprotection.* In a vessel for manual synthesis, the peptide resin (9.52 g) was treated with CH_2Cl_2/2-methylbut-2-ene/CF_3COOH 94:5:1 (80 ml, 12×2 min). The mixture obtained from this cleavage was added to a soln. of pyridine (20.2 ml) in MeOH (202 ml), evaporated, redissolved in $CHCl_3$ and washed with a 0.05M K_2SO_4/$KHSO_4$ buffer (7×20 ml, pH 1.5). The combined org. extract was dried and evaporated and the residue lyophilized from dioxane/H_2O: **7** (2.96 g, 2.85 mmol). HPLC: t_R 17.1. FAB-MS (thioglycerol): 1035(60, $[M+H]^+$), 979(10, $[M-{}^t\text{Bu}+H]^+$), 945(8, $[M-\text{Bzl}+H]^+$), 769(29, $[M-\text{Pmc}+H]^+$), 695(13), 496(20), 426(12), 392(10), 367(11), 299(12), 252(18), 227(81), 204(59, $[\text{Pmc}-SO_2+H]^+$), 147(100).

Ac-Glu-Tyr(PO$_3$H$_2$)-Leu-Arg-NH$_2$ (**9**). The phosphopeptide **9** was prepared using the *General Procedure* described above (see *b*). *Rink*-amide resin (*Novabiochem*, Läufelfingen, Switzerland; 0.55 mmol/g) was deprotected. Then, Fmoc-Arg(Pmc)-OH was attached to the resin with TPTU as condensing agent in 30 min. Fmoc-Leu-OH was then attached with TPTU in 2 h and the resin was divided in two portions. Fmoc-Tyr(PO_3H_2) was coupled with HATU (2 equiv. of the amino acid, 2 equiv. of HATU, and 6 equiv. of 1.5N $(^i\text{Pr})_2$EtN in NMP were stirred for 5 min prior to addition) and recoupled with (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (2 equiv. of the amino acid, 2 equiv. of BOP, 2 equiv. of 1.0N HOBt in NMP, and 6 equiv. of 1.5N $(^i\text{Pr})_2$EtN in NMP were stirred for 10 min prior to addition). Coupling of the N-terminal Glu was achieved using Fmoc-Glu(O^{t}Bu)-OH with TPTU, DICD/HOBt, and BOP as condensing agents. During condensation with BOP, the resin was kept at 40–50°. After completion of chain assembly, the N-terminal Fmoc group was removed and the resin was acetylated for 20 min with DMA/pyridine/Ac_2O 8:1:1. In a vessel for manual synthesis, the peptide resin was then shaken with $CF_3COOH/H_2O/(CH_2SH)_2$ 76:4:20 (9 ml) for 3 h. The crude peptide was precipitated from the orange mixture by addition of ^{t}BuOMe/petroleum ether 2:1 (210 ml), cooled, collected by centrifugation (*Sigma Labocentrifuge 3–10*, 10 min, 4200 min^{-1}), the solvent removed by decantation, and the residue dissolved in dioxane/H_2O and lyophilized. The peptide (160.1 mg) was purified by MPLC (*Merck-Lichroprep-RP-18 gel*; in 20 min from 0 to 13% *B*, 10 min at 13% *B* and in 10 min from 13 to 18% *B*; flow rate 60 ml/min). The product (93.8 mg) was dissolved in CF_3COOH and added to cold ^{t}BuOMe and stirred for 10 min. The precipitate was then collected by centrifugation and the solvent removed by decantation. The residue was triturated several times with ^{t}BuOMe and collected by centrifugation, the solvent removed by decantation, and the residue lyophilized from dioxane/H_2O to yield pure **9** (60 mg, 38%). Colorless, amorphous powder. HPLC (*Nucleosil-C$_{18}$-AB* column, in 12 min from 0 to 25% *B*): t_R 6.82. ^{31}P-NMR (121 MHz, $(CD_3)_2SO$): 2.10 (*s*). FAB-MS (thioglycerol): 723(21, $[M+Na]^+$), 701(100, $[M+H]^+$). MALDI-MS (2,6-DHA + DAHC): pos. mode: 700.6 ($[M+H]^+$); neg. mode: 697.2 ($[M-H]^-$).

Ac-Glu(O^{t}Bu)-Tyr-Leu-Arg(Pmc)-NH$_2$ (**8**). To a soln. of **7** (517 mg, 500 µmol), HOBt (136 mg, 1.00 mmol), and $(^i\text{Pr})_2$EtN (163 µl, 1.00 mmol) in DMF (25 ml), 5% NH_4OBt in DMF (6.60 ml, 2.00 mmol) [25], 5% $CuCl_2$ in DMF (7.00 ml, 2.50 mmol), and 10% TBTU in DMF (3.30 ml, 1.00 mmol) were added. After stirring for 15 min

at r.t., more 5% NH_4OBt in DMF (3.30 ml, 1.00 mmol) and 10% TBTU in DMF (3.30 ml, 1.00 mmol) were added. The dark yellow mixture was stirred for 2 h. After the addition of AcOEt (250 ml) and sat. $NaHCO_3$ soln., the light blue mixture was washed with sat. $NaHCO_3$ soln. and H_2O, the combined org. extract dried and evaporated, and the residue lyophilized from dioxane/H_2O. FC (CH_2Cl_2/MeOH 95:5) and lyophilization from dioxane/H_2O gave *Ac-Glu(O^{t}Bu)-Tyr(Bzl)-Leu-Arg(Pmc)-NH$_2$* (360 mg, 70%). Colorless solid. HPLC: t_R 20.3. FAB-MS (thioglycerol): 1033(24 $[M + H]^+$), 977(6, $[M - {}^t\text{Bu} + H]^+$), 767(13, $[M - \text{Pmc} + H]^+$), 693(5), 494(12), 251(17), 226(80), 203(48, $[\text{Pmc} - SO_2 + H]^+$), 147(100).

A soln. of Ac-Glu(O^{t}Bu)-Tyr(Bzl)-Leu-Arg(Pmc)-NH_2 (270 mg, 261 μmol) in EtOH/H_2O 9:1 (40 ml) was vigorously shaken under H_2 (1 atm, r.t.) in the presence of 10% Pd/C (29 mg). After 17 h, the catalyst was filtered off and washed with EtOH/H_2O 9:1, the filtrate evaporated, and the residue lyophilized from dioxane/H_2O. The residue obtained was suspended in AcOEt and poured into ^{t}BuOMe. The precipitate was centrifuged, decanted, triturated with additional ^{t}BuOMe, and lyophilized from dioxane/H_2O to give pure **8** (240 mg, 97%). HPLC: t_R 14.5. FAB-MS (thioglycerol): 965(21, $[M + Na]^+$), 953(83, $[M + H]^+$), 887(8, $[M - {}^t\text{Bu} + H]^+$), 677(13, $[M - {}^t\text{Bu} - \text{Pmc} + H]^+$), 603(20), 494(18), 366(11), 335(19), 297(13), 251(21), 219(27), 203(52, $[\text{Pmc} - SO_2 + H]^+$), 172(18), 147(91), 136(90).

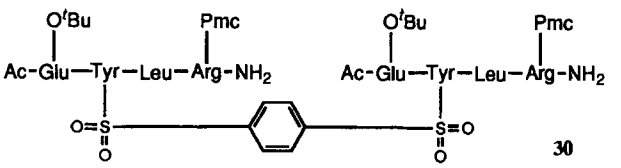
Ac-Glu(O^{t}Bu)-Tyr[SO$_2$C$_6$H$_3$(3-OH)(4-COOH)]-Leu-Arg(Pmc)-NH$_2$ (**14**). To a soln. of **8** (155 mg, 164 μmol) in *Tris* buffer (3 ml, pH 9) and dioxane (12 ml) was added solid 4-(chlorosulfonyl)-2-hydroxybenzoic acid (133 mg, 564 μmol) in 3 portions. The pH was kept within 9–10 by addition of 2N NaOH. The orange mixture was stirred for 24 h at r.t. and then neutralized to pH 7. The dioxane was evaporated and the aq. soln. dissolved in AcOEt (50 ml) and acidified with 2N H_2SO_4 to pH 4.5. The org. phase was separated, washed with 10% citric acid soln. and H_2O, dried, and evaporated. FC (CH_2Cl_2/MeOH 9:1, 9:2 + 5 drops of 1% HCOOH soln., 9:3 + 10 drops of 1% HCOOH soln.) and lyophilization afforded pure, solid **14** (151 mg, 80%). HPLC: t_R 15.9. ^{252}Cf-PD-MS (soln. in CF_3CH_2OH): 1144.9($[M + H]^+$), 1090.3($[M - {}^t\text{Bu} + H]^+$), 944.9($[M - \text{spacer} + H]^+$), 879.7($[M - \text{Pmc} + H]^+$). MALDI-MS (2,5-DHB): pos. mode: 1168($[M + Na]^+$), 1146($[M + H]^+$), neg. mode 1145 ($[M - H]^-$).

Ac-Glu-Tyr[SO$_2$C$_6$H$_3$(3-OH)(4-COOH]-Leu-Arg-NH$_2$ (**18**). For 3 h, **14** (40.1 mg, 200 μmol) was treated with $CF_3COOH/H_2O/(CH_2SH)_2$ 76:4:20 (1.5 ml). The mixture was worked up as described for **9** to give **18** (26.6 mg, 92%). Colorless powder. HPLC: t_R 9.91. ^{1}H-NMR (400 MHz, $(CD_3)_2SO$): *Table 2*. ^{13}C-NMR (101 MHz, $(CD_3)_2SO$): *Table 3*. FAB-MS (glycerol/H_2O): 821(5, $[M + H]^+$), 659(8), 621(100, $[M - \text{spacer} + H]^+$), 429(9) 337(58), 245(29), 223(55), 158(20), 172(18), 136(52), 86(53), 70(71).

Ac-Glu(O^{t}Bu)-Tyr[SO$_2$C$_6$H$_4$(4-SO$_3$H)]-Leu-Arg(Pmc)-NH$_2$ (**15**). As described for **14**, with **8** (248 mg, 263 μmol), dioxane (100 ml), *Tris* buffer (25 ml, pH 9), and solid benzene-1,4-disulfonyldichloride (**11**; 170 mg, 617 μmol; prepared according to [32] [33]). Purification by prep. TLC (CH_2Cl_2/MeOH 9:2; extraction with CH_2Cl_2/MeOH 80:20) and lyophilization from dioxane/H_2O yielded amorphous **15** (164.9 mg, 53%) and the sulfonic acid diester **30** (62.2 mg, 22%).

Data of **15**: HPLC: t_R 14.0. ^{252}Cf-PD-MS (soln. in H_2O/AcOH 1:1): pos. mode: 1187.7($[M + Na]^+$), 1165.7($[M + H]^+$), 943, 346, 203($[\text{Pmc} - SO_2 + H]^+$), 147; neg. mode: 1163.2($[M - H]^-$), 221.2, 204.0, 156.1.

Data of **30**: HPLC: t_R 19.3. MALDI-MS (α-CHC + DAHC): pos. mode: 2091.2($[M + H]^+$); neg. mode: 2087.8($[M - H]^-$).



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Ac-Glu-Tyr[SO$_2$C$_6$H$_4$(4-SO$_3$H)]-Leu-Arg-NH$_2$ (**19**). As described for **18**, with **15** (40.7 mg, 34.9 μmol) and $CF_3COOH/H_2O/(CH_2SH)_2$ 76:4:20 (1.5 ml). Workup as described for **9** gave **19** (24.8 mg, 84%). HPLC: t_R 7.69. ^{252}Cf-PD-MS (soln. in H_2O/AcOH 1:1): pos. mode; 843.4($[M + H]^+$), 621.1($[M - \text{spacer} + H]^+$), 516.3, 342.5, 228.5, 207.1; neg. mode: 840.2 ($[M - H]^-$), 353.9, 221.1, 156.1.

REFERENCES

[1] A. Ullrich, J. Schlessinger, *Cell* **1990**, *61*, 203.
[2] S. A. Aaronson, *Science* **1991**, *254*, 1146.
[3] X. Lia, T. Pawson, *Recent Prog. Horm. Res.* **1994**, *49*, 149.
[4] G. Carpenter, *Ann. Rev. Biochem.* **1987**, *56*, 881.
[5] P. J. Bertics, G. N. Gill, *J. Biol. Chem.* **1985**, *260*, 14642.
[6] T. Hunter, J. A. Cooper, *Ann. Rev. Biochem.* **1985**, *54*, 897.
[7] J. Schlessinger, *Biochemistry* **1988**, *27*, 3119.
[8] Y. Yarden, A. Ullrich, *Ann. Rev. Biochem.* **1988**, *57*, 443.
[9] J. Schlessinger, A. Ullrich, *Neuron* **1992**, *9*, 383.
[10] L. C. Cantley, K. R. Auger, C. Carpenter, B. Duckworth, A. Graziani, R. Kapeller, S. Soltoff, *Cell* **1991**, *64*, 281.
[11] C.-J. Chang, R. L. Gaehlen, *J. Nat. Prod.* **1992**, *55*, 1529.
[12] P. Workman, V. G. Brunton, D. J. Robins, *Semin. Cancer Biol.* **1992**, *3*, 369.
[13] T. R. Burke, Jr., *Drugs of the Future* **1992**, *17*, 119.
[14] D. W. Fry, *Exp. Opin. Invest. Drugs* **1994**, *3*, 577.
[15] A. Levitzki, A. Gazit, *Science* **1995**, *267*, 1782.
[16] P. Traxler, N. Lydon, *Drugs of the Future* **1995**, *20*, 1261.
[17] D. R. Knighton, D. L. Cadena, J. Zheng, L. F. Ten Eyck, S. S. Taylor, J. M. Sowadski, G. N. Gill, *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5001.
[18] P. M. Traxler, O. Wacker, H. L. Bach, J. F. Geissler, W. Kump, T. Meyer, U. Regenass, J. L. Roesel, N. Lydon, *J. Med. Chem.* **1991**, *34*, 2328.
[19] S. Peterli, R. Stumpf, M. Schweizer, U. Séquin, H. Mett, P. Traxler, *Helv. Chim. Acta* **1992**, *75*, 696.
[20] S. Peterli, D. Hubmann, U. Séquin, H. Mett, P. Traxler, *Helv. Chim. Acta* **1994**, *77*, 59.
[21] R. B. Pearson, B. E. Kemp, *Methods Enzymol.* **1991**, *200*, 62.
[22] E. Atherton, R. C. Sheppard, in 'Solid Phase Peptide Synthesis – A Practical Approach', Eds. D. Rickwood and B. D. Hames, IRL Press at Oxford University Press, Oxford, 1989.
[23] B. Riniker, A. Flörsheimer, H. Fretz, P. Sieber, B. Kamber, *Tetrahedron Lett.* **1993**, *49*, 9307.
[24] R. Knorr, A. Trzeciak, W. Bannwarth, D. Gillessen, *Tetrahedron Lett.* **1989**, *30*, 1927.
[25] R. Briand, S. Kläusler, G. Rossé, H. Fretz (*Ciba-Geigy AG*, Basel), unpublished results.
[26] S.-T. Chen, S.-H. Wu, K.-T. Wang, *Synthesis* **1989**, 37.
[27] C. Somlai, G. Szókán, L. Baláspiri, *Synthesis* **1992**, 285.
[28] T. Miyazawa, T. Donkai, T. Yamada, S. Kuwata, *Int. J. Pept. Protein Res.* **1992**, *40*, 49.
[29] E. A. Ottinger, L. L. Shekels, D. A. Bernlohr, G. Barany, *Biochemistry* **1993**, *32*, 4354.
[30] L. A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397.
[31] L. A. Carpino, A. El.-Faham, F. Albericio, *Tetrahedron Lett.* **1994**, *35*, 2279.
[32] H. Meerwein, G. Dittmar, R. Göllner, K. Hafner, F. Mensch, O. Steinfort, *Chem. Ber.* **1957**, *6*, 841.
[33] B. I. Karavaev, S. P. Starkov, *J. Gen. Chem. USSR (Engl. Transl.)* **1957**, *27*, 863.
[34] A. K. Saha, P. Schultz, H. Rapoport, *J. Am. Chem. Soc.* **1989**, *111*, 4856.
[35] E. H. Farmer, J. Kracovski, *J. Chem. Soc.* **1927**, 680.
[36] A. Devos, J. Remion, A.-M. Frisque-Hesbain, A. Colens, L. Ghosez, *J. Chem. Soc., Chem. Commun.* **1979**, 1180.
[37] B. Haveaux, A. Deboker, M. Rens, A. R. Sidani, J. Toye, L. Ghosez, *Org. Synth. Coll.* **1988**, *6*, 282.
[38] D. Medzihradszky, S. L. Chen, G. L. Kenyon, B. W. Gibson, *J. Am. Chem. Soc.* **1994**, *116*, 9413.
[39] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923.
[40] E. McGlynn, M. Becker, H. Mett, S. Reutener, R. Cozens, N. B. Lydon, *Eur. J. Biochem.* **1992**, *207*, 265.
[41] E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.* **1970**, *34*, 595.