Synthesis and Enzymatic Hydrolysis of Esters, Constituting Simple Models of Soft Drugs

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One way to minimise systemic side effects of drugs is to design molecules, soft drugs, in such a way that they are metabolically inactivated rapidly after having acted on their pharmacological target. Hydrolases (esterases, peptidases, lipases, glycosidases, *etc.*) are enzymes well suited to use for drug inactivation since they are ubiquitously distributed. Insertion of ester bonds susceptible to enzymatic cleavage may represent one approach to make the action of a drug more restricted to the site of application.

The present study describes the chemical synthesis of fourteen model compounds comprising a bicyclic aromatic unit connected by an ester-containing bridge to another aromatic ring. Initial attempts to define a) the tissue selectivity of the hydrolytic metabolism and b) the molecular structural factors affecting the rate of enzymatic ester cleavage are presented.

The data show that human and rat liver fractions were more active than human duodenal mucosa and human blood leukocytes at hydrolysing the compounds. The rank order of the compounds was, however, very similar in the different biological systems. Commercially available pig liver carboxyl esterase and cholesterol esterase both reasonably well predict the rank order in the tissue fractions.

Key words soft drug; enzymatic hydrolysis; aromatic ester; esterase

Metabolic/pharmacokinetic aspects are important in the design of new drugs. In addition, modulation of the chemical structure of known drugs may result in kinetic properties yielding improved therapeutic ratios.

Drugs that are intentionally designed to undergo rapid and efficient inactivation after having exerted effects at the site of action are termed soft drugs. Undesired systemic side effects can thereby be avoided. The soft drug principle is important, for example, for inhaled antiasthma glucocorticoids such as budesonide[1] and fluticason.[2] These substances have antiinflammatory actions in the airways, but are effectively inactivated in the liver and/or in the intestine, and the systemic side effects from the swallowed part of the dose and from the part of the inhaled dose entering into the systemic circulation, are minimised.[3] Furthermore, budesonide was recently launched as a treatment for inflammatory bowel diseases (IBD), with reduced side effects compared to other steroids.[4,5]

The two drugs mentioned above as examples, budesonide and fluticason, are inactivated by the cytochrome P450-system. Other enzymes of interest, with regard to soft drug design, are the hydrolases, for example, esterases and lipases. Flestolol, an ultrashort-acting β-blocker intended for the treatment of myocardial dysfunction, constitutes an example of a soft drug which is inactivated by esterases.[6,7] Soft drugs, relying on inactivation catalysed by esterases/lipases, could also be effective against IBD. Efficient inactivation of a topically active IBD drug in the liver, blood or perhaps in the deeper layers of the gut mucosa should deliver a beneficial effect/side-effect ratio.

The preconditions in the design of soft drugs are that hydrolysable functions are allowed to be inserted into the structure and that the pharmacological activity of the original drug is retained after this manipulation. Furthermore, the metabolites formed after enzymatic hydrolysis must be considerably less pharmacologically active than the soft drug itself. Hydrolytic enzymes such as carboxyl esterases are present in most, if not all, tissues, although the liver microsomal fraction is considered to be the richest source.[8,9] The ubiquitous distribution of esterases may be seen as an advantage since the soft drug thus can be inactivated in most organs. At the same time, this presents a potential obstacle, since creation of structures that are esterase substrates, but retain sufficient activity in target effect organs, is a challenging task.

We are interested in soft drugs of immunosuppressing/antiinflammatory agents, comprising bicyclic aromatic units linked to another aromatic ring as core structures. We intend to connect the ring systems by ester-containing bridges, making the drugs targets for esterases. As a prelude to a more comprehensive medicinal chemistry program, we required information on the factors determining the rate of hydrolysis and decided therefore to explore the impact of a) the orientation of the ester function, b) the length of the ester bridge, and c) the structure of the aromatic moiety. Furthermore, we wanted to elucidate a) whether tissue-selective hydrolysis could be achieved, and b) whether pig liver carboxyl esterase (PLE) and/or cholesterol esterase could provide simple assays for adequate prediction of the ranking of rates of hydrolysis in human tissues. In this report, we present the syntheses of the esters **2**—**14** (compound **1** is commercially available), and a comparison of the rates of hydrolysis of these compounds catalysed by PLE, cholesterol esterase from bovine pancreas, rat and human liver S9 fractions, human duodenal homogenate, and human blood leukocytes.

Chemical Synthesis Compounds **2**—**6** were smoothly prepared by treatment of phenol, 2-naphthol, benzyl alcohol and 2-naphthalenemethanol with the proper acid chlorides and with triethylamine as a base. For the preparation of the carboxylic acids **7**—**9** and **12** more

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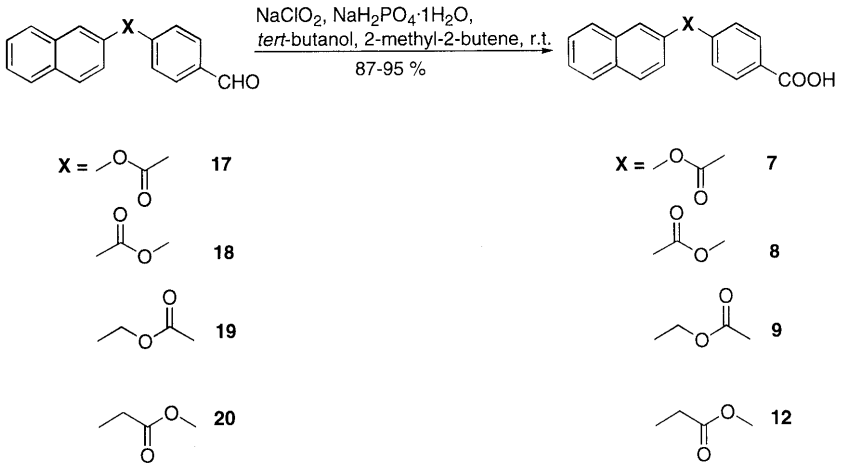
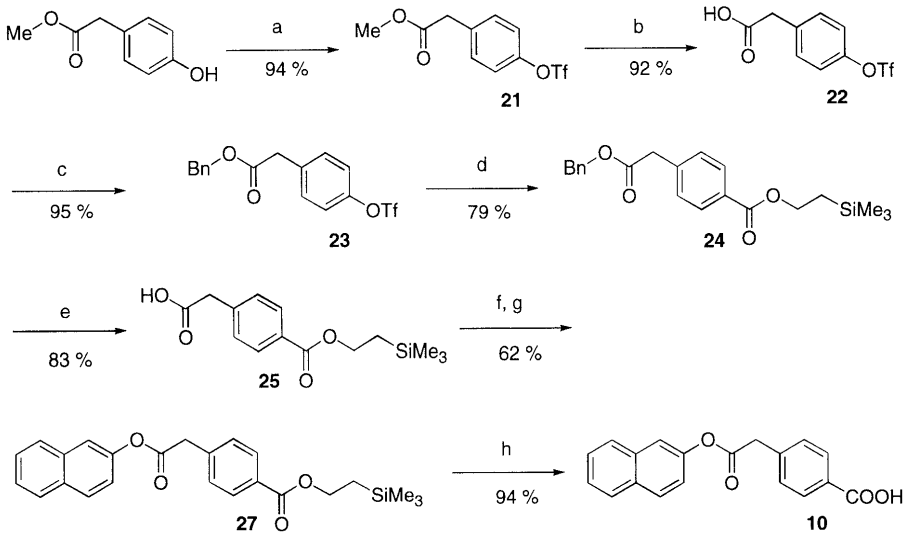


Chart 1



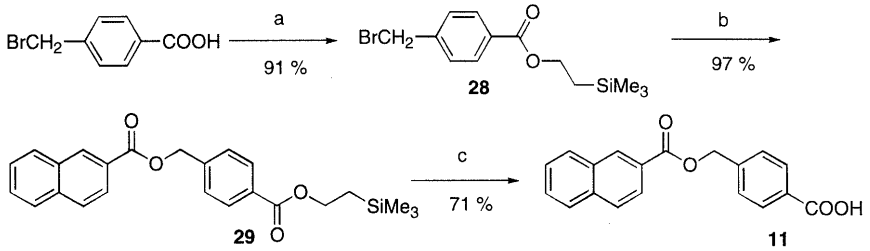
Reagents: (a) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, 0 °C; (b) TFA, reflux; (c) benzyl bromide, K_2CO_3, acetone, reflux; (d) CO (1 atm), $Pd(OAc)_2$, 1,3-bis(diphenylphosphino)propane (dppp), 2-(trimethylsilyl)ethanol, Et_3N, DMF, 80 °C; (e) H_2 (1 atm), 10% Pd/C, THF; (f) oxalyl chloride, toluene; (g) 2-naphthol, Et_3N, diethyl ether; (h) NaI, TMSCl, CH_3CN, reflux.

Chart 2

elaborate procedures were needed. For the preparation of **7** and **9** we employed 4-formylbenzoic acid as starting material, which, after conversion to the acid chloride, was esterified with 2-naphthol and 2-naphthalenemethanol, respectively. The subsequent oxidations of the aldehyde functions were conducted with sodium chlorite employing 2-methyl-2-butene as a scavenger[10-12] as depicted in Chart 1. The carboxylic acids **8** and **12** were prepared according to the same method with 4-hydroxybenzaldehyde as the precursor.

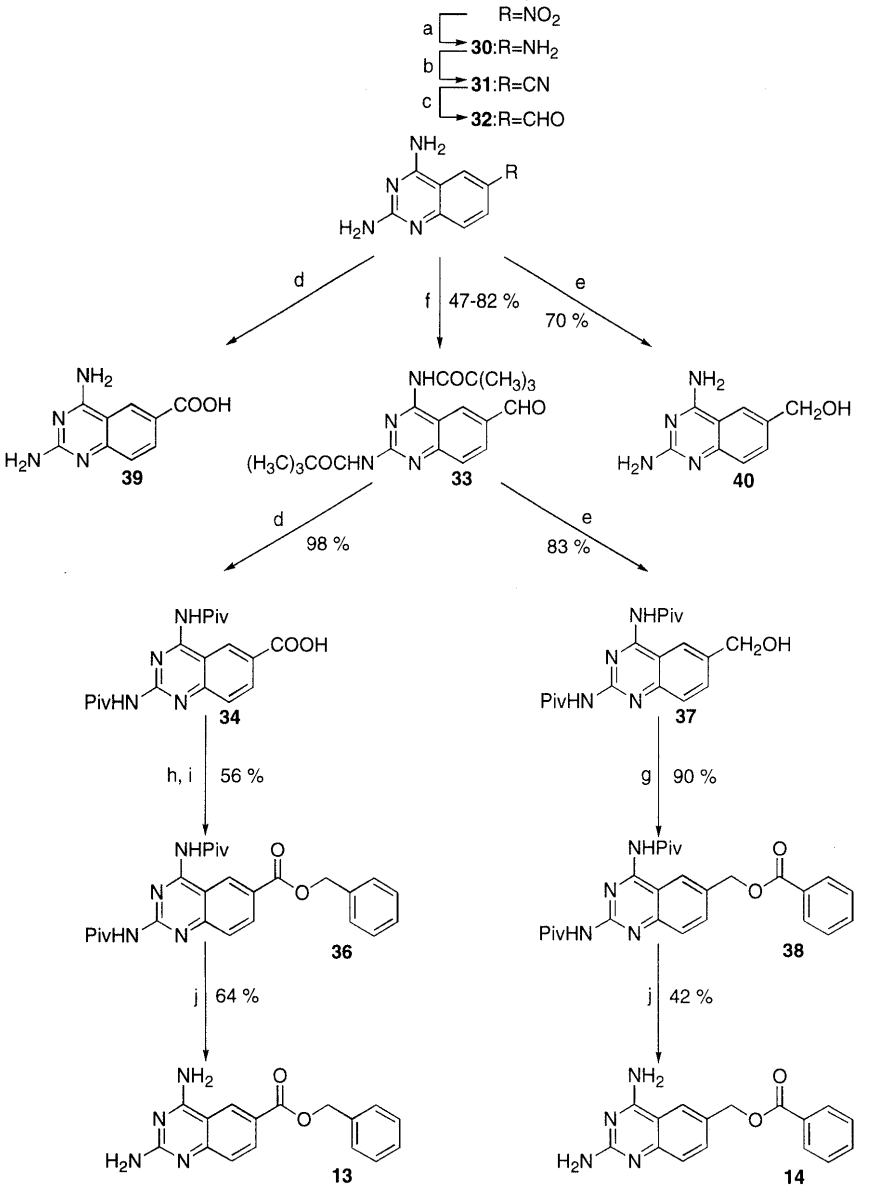
For the preparation of **10**, we followed the reaction sequence outlined in Chart 2 starting from commercially available methyl (4-hydroxy)phenylacetate. We used the 2-(trimethylsilyl)ethyl ester as a protecting group, which should allow a selective deprotection in the last step of the sequence. The triflate **21** was prepared[13] and the methylester was hydrolysed with trifluoroacetic acid (TFA)[14,15] to afford the carboxylic acid **22**. Conversion of **22** to **10**, *via* direct esterification with 2-naphthol and subsequent palladium-catalysed carbonylation[16,17] in the presence of 2-(trimethylsilyl)ethanol, failed due to a concomitant predominant transesterification under the conditions required for the carbonylation to occur. The carboxylic acid group of compound **22** was therefore first protected by benzylation to give **23**, after which the palladium-mediated carbonylative esterification was conducted to furnish the diester **24**. Debenzylation and reaction of **25** with oxalyl chloride and 2-naphthol afforded **27**, which finally was transformed to **10** by selective ester cleavage, using trimethylsilyl chloride/sodium iodide.[18] Deprotection of the 2-(trimethylsilyl)ethyl group of **27** could not be accomplished with tetrabutylammonium fluoride (TBAF). A fast cleavage of the 2-naphthylester linkage was observed with this reagent, reflecting the propensity of the 2-naphthylester group to undergo hydrolysis.

The carboxylic acid **11** was synthesised as outlined in Chart 3. Reaction of 4-bromomethylbenzoic acid with 2-(trimethylsilyl)ethanol with the traditional ester-coupling reagent *N,N'*-dicyclohexylcarbodiimide provided the



Reagents: (a) 2-(trimethylsilyl)ethanol, *N*,*N*′-dicyclohexylcarbodiimide, 4-dimethylaminopyridine, CH_2Cl_2, r.t.; (b) 2-naphthoic acid, K_2CO_3, acetone, r.t.; (c) TBAF, 1 M in THF, r.t.

Chart 3



Reagents: (a) H_2 (4 atm), 10% Pd/C, AcOH, DMF; (b) $NaNO_2$, KCN, $Cu(II)SO_4 \times 5H_2O$, (c) Ni–Al alloy, 75% HCO_2H, reflux; (d) $NaClO_2$, $NaH_2PO_4 \times 1H_2O$, *tert*-butanol, 2-methyl-2-butene, r.t.; (e) $NaBH_4$, MeOH; (f) pivalic anhydride, DMF, 80 °C; (g) benzoyl chloride, Et_3N, CH_2Cl_2; (h) oxalyl chloride, toluene; (i) benzyl alcohol, Et_3N, CH_2Cl_2; (j) NH_3 (aq.), dioxane.

Chart 4

ester **28**. Selective deprotection of **29** in this system could be achieved with TBAF, which furnished **11**.

The 2,4-diaminoquinazoline esters **13** and **14** were prepared from the 2,4-diaminoquinazoline-6-carbaldehyde **32** as shown in Chart 4. This aldehyde **32** was obtained after reduction of 2,4-diamino-6-nitroquinazoline to the 2,4,6-triaminoquinazoline **30**, followed by diazotation to the nitrile **31** and final reduction/hydrolysis,

via a slightly modified version of previously described methods.[19,20] A screening of a series of protection groups revealed that the pivaloyl group[21-23] provided suitable protection of the amino functions in the subsequent transformations (Chart 4). Though it is reported that direct pivaloylation of similar compounds containing a formyl group should not give satisfactory results,[23] we succeeded with this one step reaction in very nice yields. Oxidation of **33**, conversion to the acid chloride and treatment with benzyl alcohol gave **36** while reduction of **33** with sodium borohydride and reaction with benzoyl chloride furnished the ester **38**. Deprotection could be accomplished with aqueous ammonia in dioxane,[24] eventually delivering **13** and **14**, respectively. However, we quenched the reaction at a stage where a small amount of monopivaloyl-protected amine still remained, since the competing ester cleavage was becoming significant. The metabolites **39** and **40** were prepared from the aldehyde **32** by oxidation and reduction, respectively.

Results and Discussion

Enzymatic Hydrolysis The rates of hydrolysis for the fourteen substrates in six different incubation systems are presented in Table 1. The relative rates of hydrolysis are within parantheses. The rate of hydrolysis for compound **1** in each system was defined as 100%.

PLE, a commercially available enzyme, hydrolyses all of the substrates (Table 1). The substrate selectivities of PLE and cholesterol esterase were similar, but not identical. Rat liver and human liver S9 fractions were, in most cases, more active than a homogenate of human duodenal mucosa. Hydrolysis of the compounds was also detected in sonicated human blood leukocytes. The substrate selectivity was relatively similar in all of the tissue fractions used. Chemical hydrolysis was negligible for all compounds.

The following structural features of the substrates appear to affect the rates of hydrolysis:

a) Orientation of the Ester Bond: Compound **1**, yielding 2-naphthol as a metabolite, was more rapidly hydrolysed than **2**, yielding phenol, in all incubation systems. This is consistent with the fact that 2-naphthoate is a better leaving group than phenolate.

b) Elongation of the Bridge by a Methylene Group: When a methylene group was inserted into the bridge between the aromatic systems, those substrates giving 2-naphthol and phenol as metabolites (**4**, **6**) were hydrolysed faster than substrates resulting in 2-naphthalenemethanol and benzyl alcohol (**3**, **5**). Thus, insertion of the methylene group between the aromatic system and the alcohol function retarded the hydrolysis in all systems (*cf.* **1** *vs.* **3**, **2** *vs.* **5**) while insertion of the methylene group between the aromatic system and the carboxylic acid function (*cf.* **1** *vs.* **4**, **2** *vs.* **6**) affected the rates of hydrolysis in a more marginal and less predictable manner. One might have expected that the elongation of the bridge would have increased the rate of hydrolysis,[7] since the ester bond would be more accessible to the enzymes.

c) Introduction of a Carboxylic Acid Function: Introduction of a carboxylic acid function on the phenyl ring markedly retarded the rates of hydrolysis (**1**—**6** *vs.* **7**—**12**). This effect is expected, since carboxylic acids are products of hydrolysis and are repelled from the active site.

d) Substitution of 2,4-Diaminoquinazoline for the Naphthalene Ring: Replacement of the naphthalene ring by 2,4-diaminoquinazoline afforded poor hydrolase substrates (*cf.* **3** *vs.* **14**, **5** *vs.* **13**).

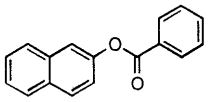
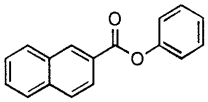
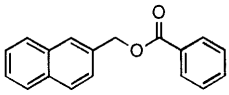
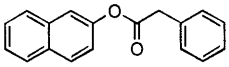
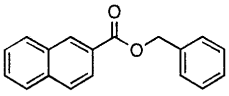
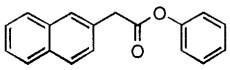
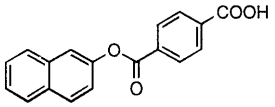
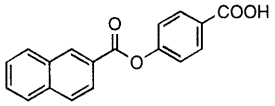
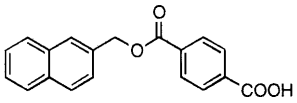
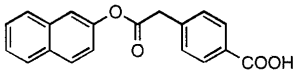
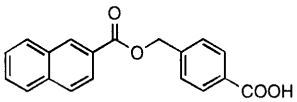
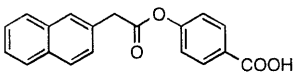
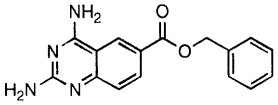
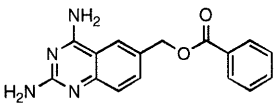
The rates of hydrolysis were compared in different biological systems. PLE was selected since it is an easily available representative of the enzyme family "unspecific carboxyl esterases". These enzymes are ubiquitously distributed, but liver microsomes are the richest source.[8,9] Cholesterol esterase was selected since it is a type of hydrolase to which every orally taken drug will be exposed in the intestinal lumen. Further, hydrolyses in liver S9 fractions from rat and human were studied since these are rich sources of hydrolases involved in first-pass metabolism of esters. Human duodenal mucosa homogenate was used since this tissue, next to the luminal content of the intestinal system, is the first barrier a drug has to penetrate following oral intake. Human blood leukocytes were chosen since these cells may be targets for immunosuppressive drugs.

Several investigations have been devoted to the role of steric and electronic factors in determining the rate of esterase-catalysed hydrolysis. Relevant examples for the present investigation include studies of the hydrolyses of esters of benzoic acid,[25] salicylic acid,[26,27] nicotinic acid,[28,29] nipecotic acid,[30] corticosteroids,[31] phenetsal,[32] and pilocarpine.[33] One study on the debenzoylation of **1** in rat liver and mammary gland microsomes has been reported,[34] while monoaromatic systems linked to monoaromatic systems by ester bonds have been examined more frequently.[25,28,29,32,35] A recent study of nicotinate esters showed that phenyl nicotinate was hydrolysed at a higher rate than benzyl nicotinate in rat liver subcellular fractions.[29] This coincides well with our observations in all of the systems tested, namely that reactions leading to phenol or 2-naphthol occur at a higher rate than reactions leading to 2-naphthalenemethanol or benzyl alcohol, respectively, as is clear from a comparison of **1** *vs.* **3** and **2** *vs.* **5**. An X-ray structure of PLE has not been reported but several topographical models of the active site have been postulated.[36] In our initial experiments we found that phenyl benzoate was hydrolysed twice as fast as 2-naphthyl benzoate (**1**) by PLE. Phenyl benzoate fits well into the model described by Toone *et al.*,[36] but the more sterically demanding 2-naphthyl derivatives **1**—**6** can also be accommodated.

It is known that introduction of a negatively charged carboxyl group in close proximity to the cleavage site provides poor substrates for esterases.[25-27,31] Thus, benzoylglycolic acid is essentially inert in human plasma, while the lipophilic benzyl benzoate is a good substrate.[25] A comparison of **1**—**6** with **7**—**12** demonstrates that a carboxyl group in a more remote position still exerts an impact on the rate of hydrolysis.

We selected **3** and **5**, the two esters found to be least prone to hydrolysis in most media, as reference structures and substituted 2,4-diaminoquinazoline for naphthalene. 2,4-Diaminoquinazoline contains a 2,4-diaminopyrimidine unit, a common fragment in many drugs[37] (*e.g.*, methotrexate). The nitrogen heterocycles were found to

Table 1. Rates of Hydrolysis for Compounds **1**—**14**

Compound	No.	Pig liver esterase[a]	Cholesterol esterase[b]	Human duodenal mucosa[c]	Human liver S9[c]	Rat liver S9[c]	Human leukocytes[d]
	**1**	170 (100)	540 (100)	39 (100)	320 (100)	510 (100)	0.46 (100)
	**2**	100 (60)	240 (43)	26 (68)	170 (54)	170 (34)	0.14 (31)
	**3**	120 (70)	60 (11)	4.6 (12)	69 (22)	150 (29)	0.11 (24)
	**4**	150 (88)	1240 (227)	18 (46)	160 (51)	440 (86)	0.65 (141)
	**5**	36 (23)	26 (4.7)	0.7 (1.8)	41 (13)	46 (8.9)	0.034 (7.3)
	**6**	130 (78)	340 (61)	4.7 (12)	220 (68)	730 (143)	0.26 (56)
	**7**	2.1 (1.2)	n.q.	0.3 (0.6)	13 (3.9)	100 (20)	0.006 (1.3)
	**8**	36 (21)	10 (1.8)	0.7 (1.7)	9.1 (2.8)	11 (2.1)	0.014 (3.1)
	**9**	0.5 (0.3)	n.q.	n.q.	n.q.	4.1 (0.9)	n.q.
	**10**	2.6 (4.0)	n.q.	0.2 (1.1)	3.1 (1.2)	290 (59)	0.025 (8.0)
	**11**	8.6 (13)	n.q.	n.q.	0.4 (0.2)	5.4 (1.1)	0.003 (1.0)
	**12**	11 (6.4)	13 (2.3)	0.5 (1.2)	11 (3.4)	27 (5.1)	0.12 (37)
	**13**	0.1 (0.1)	n.q.	n.q.	n.q.	2.5 (0.4)	n.q.
	**14**	4.6 (6.9)	n.q.	0.6 (1.5)	2.4 (0.8)	2.9 (0.6)	n.q.

Relative rates are shown within parentheses. The rate of hydrolysis for compound **1** in each system was defined as 100%. n.q.: not quantifiable *a*) Pig liver esterase: nmol/ml/min (calculated to a dilution of 1/8000 of the enzyme suspension obtained from Sigma). *b*) Cholesterol esterase: nmol/ml/min at an enzyme concentration of 1 mg/ml. *c*) Human duodenal mucosa, human liver S9 and rat liver S9: nmol/ml/min at a protein concentration of 1 mg/ml. *d*) Human leukocytes: nmol/ml/min at 10^6 cell/ml.

be poor substrates (**13**, **14**). The considerably higher reaction rate of hydrolysis found with **3** as a substrate, as compared with **14**, indicates that the debenzoylation and regeneration of a catalytically active enzyme is not rate-determining.

Nitrogen atoms in aromatic systems close to the cleavage site are generally well tolerated[28,29,33] while adjacent alkylamino groups, which are protonated at physiological pH, provide inferior substrates for carboxyl esterases.[28] Compounds **13** and **14** are not likely to be protonated to a large extent in the media used in the enzyme assay and the reason for the poor hydrolytic activity as compared to the neutral substrates is not clear.

The work presented in this paper originates from considerations of the pharmacology and pharmacokinetics of methotrexate. Methotrexate is a cytotoxic agent, but is also an immunosuppressive drug when given in lower doses. Methotrexate is active against various autoimmune diseases, such as rheumatoid arthritis,[38-40] asthma[41,42] and IBD.[43,44] Because of the severe side effects, the clinical use of the drug is limited. We hoped that soft drugs based on methotrexate and related analogues would represent attractive alternatives in the treatment of IBD. Only salicylate and paracetamol were detected in the plasma of human volunteers after oral administration of benorylate ((4-acetamidophenyl)-2-acetyloxybenzoate),[32] demonstrating that compounds related to those studied in the present investigation can be efficiently hydrolysed *in vivo*. However, although no *in vivo* experiments were performed with **13** and **14**, the very slow rate of hydrolysis *in vitro* suggests that esters comprising a 2,4-diaminopyrimidine ring are not suitable as soft drugs.

Conclusion

A very low tissue specificity is achieved with the substrates employed in this study. For predictions of rates of hydrolysis of ester analogues in tissue-derived fractions, it seems that both PLE and cholesterol esterase constitute relevant test systems. The 2,4-diaminoquinazolines were found to be poor substrates in the systems tested.

Experimental

Melting points (uncorrected) were determined in open glass capillaries on an Electrothermal apparatus. Infrared (IR) spectra were recorded on a Perkin-Elmer 1605 FT-IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-EX270 spectrometer at 270 and 67.8 MHz, respectively and a JEOL JNM-EX400 spectrometer at 400 and 100 MHz, respectively. Tetramethylsilane was used as internal standard. Low-resolution electron-impact MS were measured at an ionisation potential of 70 eV. The mass detector was interfaced with a gas chromatograph equipped with an HP-1 (25 m × 0.20 mm) column. Thin-layer chromatography (TLC) was performed by using aluminium sheets precoated with silica gel 60 F_{254} (0.2 mm) type E; Merck, and was visualised with UV light. Plates precoated with silica gel 60 F_{254} (2.0 mm); Merck, were used for preparative TLC. Column chromatography was conducted on silica gel S (230—400 mesh; Riedel-deHaën), silica gel 60 (230—400 mesh; Merck) or silica gel 60 (70—230 mesh; Merck), unless otherwise noted. In a few cases, columns with aluminium oxide 90 (70—230 mesh; Merck) were used. The elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden or Analytische Laboratorien, Gummersbach, Germany and were within ±0.4% of the calculated values. The high-resolution MS analyses (HR-MS) were performed by Einar Nilsson, Instrumentstationen, Kemicentrum, Lund, Sweden. Compound **1** was purchased from Lancaster 93/94 (3075). All commercial chemicals were used without further purification, unless otherwise noted.

Phenyl 2-Naphthoate (2)[45] 2-Naphthoyl chloride (169 mg, 0.89 mmol) in diethyl ether (3 ml) was added dropwise to a precooled mixture (0 °C) of phenol (100 mg, 1.1 mmol) and triethylamine (0.15 ml, 1.1 mmol) in diethyl ether (3 ml). The reaction mixture was allowed to reach room temperature overnight and was thereafter partitioned between diethyl ether and water. Washing of the organic phase with aqueous saturated $NaHCO_3$, drying ($MgSO_4$), filtration and concentration *in vacuo* gave the crude ester, which was purified by flash chromatography [diethyl ether : petroleum ether (1 : 4)], followed by purification on alumina columns [diethyl ether : petroleum ether (1 : 4) and diethyl ether, respectively], providing 149 mg (68%) as a white solid, mp 96—97 °C (lit.[45] 95—96 °C). IR (KBr) cm^{-1}: 1734 (ester). ^{1}H-NMR ($CDCl_3$) δ: 7.23—8.20 (m, 11H), 8.79 (app s, 1H). ^{13}C-NMR ($CDCl_3$) δ: 121.7 (2C), 125.4, 125.9, 126.7, 126.8, 127.8, 128.3, 128.6, 129.5 (2C), 131.8, 131.9, 132.4, 135.8, 151.0, 165.3.

2-Naphthalenemethyl Benzoate (3) Compound **3** was synthesised as described for **2**, using benzoyl chloride (0.13 ml, 1.1 mmol), 2-naphthalenemethanol (200 mg, 1.3 mmol) and triethylamine (0.18 ml, 1.3 mmol). Extraction with water, drying of the organic phase ($MgSO_4$), filtration and concentration under reduced pressure yielded an oil, which was purified on an alumina column (diethyl ether), affording 261 mg (89%) of the title compound, mp 62—63 °C. IR (KBr) cm^{-1}: 1709 (ester). ^{1}H-NMR ($CDCl_3$) δ: 5.53 (s, 2H), 7.41—8.12 (m, 12H). ^{13}C-NMR ($CDCl_3$) δ: 66.9, 125.9, 126.29 (2C), 126.32 (2C), 127.3, 127.4, 127.7, 128.0, 128.4 (2C), 129.7, 130.1, 133.1, 133.2, 133.4, 166.5. *Anal.* Calcd for $C_{18}H_{14}O_2$: C, 82.4; H, 5.4. Found: C, 82.3; H, 5.5.

2-Naphthyl Phenylacetate (4)[46] Compound **4** was prepared as described for the synthesis of **2**, using phenylacetyl chloride (0.20 ml, 1.53 mmol), 2-naphthol (200 mg, 1.39 mmol) and triethylamine (0.21 mmol). The mixture was diluted with diethyl ether (30 ml) and extracted with water (20 ml), aqueous saturated $NaHCO_3$ (20 ml) and water (20 ml). Drying ($MgSO_4$), filtration and concentration *in vacuo* and purification by flash chromatography [ethyl acetate : pentane (1 : 8)], afforded 297 mg (82%) of pure ester, mp 81—82 °C (lit.[46] 87 °C). IR (KBr) cm^{-1}: 1758 (ester). ^{1}H-NMR ($CDCl_3$) δ: 3.90 (s, 2H), 7.16—7.83 (m, 12H). ^{13}C-NMR ($CDCl_3$) δ: 41.5, 118.4, 121.0, 125.7, 126.6, 127.4, 127.6, 127.7, 128.8 (2C), 129.3 (3C), 131.5, 133.5, 133.7, 148.4, 170.2.

Benzyl 2-Naphthoate (5)[47] Compound **5** was synthesised from 2-naphthoyl chloride (2.0 g, 10.5 mmol), benzyl alcohol (1.1 ml, 11.1 mmol) and triethylamine (1.5 ml, 11.1 mmol) as described above for the synthesis of **2**. Extraction with water, drying of the organic phase ($MgSO_4$), filtration and concentration under reduced pressure afforded a solid, which was recrystallised from ethanol and diethyl ether : petroleum ether (1 : 20), furnishing 2.0 g (73%) of pure ester, mp 63.5—64 °C (lit.[47] 63—63.5 °C). IR (KBr) cm^{-1}: 1706 (ester). ^{1}H-NMR ($CDCl_3$) δ: 5.43 (s, 2H), 7.35—8.09 (m, 11H), 8.64 (app s, 1H). ^{13}C-NMR ($CDCl_3$) δ: 66.8, 125.3, 126.6, 127.4, 127.7, 128.2, 128.3 (2C), 128.6 (2C), 129.4, 131.2, 132.5, 135.6, 136.1, 166.6.

2-Naphthylacetyl Chloride (15)[48] A mixture of 2-naphthylacetic acid (5.0 g, 26.9 mmol) and $SOCl_2$ (9.0 ml) was refluxed until the reaction mixture turned brown and clear. IR indicated complete consumption of the starting material (1810 cm^{-1}). After evaporation of $SOCl_2$, the solid was stored under N_2.

Phenyl 2-Naphthylacetate (6) Compound **6** was synthesised from **15** (207 mg, 1.0 mmol), phenol (100 mg, 1.1 mmol) and triethylamine (0.15 ml, 1.1 mmol) as described above for the synthesis of **2**. Work-up as described for **2** and purification by flash chromatography [diethyl ether : petroleum ether (1 : 5)] yielded 212 mg (80%) of the pure ester, mp 107—108 °C. IR (KBr) cm^{-1}: 1758 (ester). ^{1}H-NMR ($CDCl_3$) δ: 4.02 (s, 2H), 7.04—7.87 (m, 12H). ^{13}C-NMR ($CDCl_3$) δ: 41.6, 121.4 (2C), 125.9, 126.0, 126.2, 127.2, 127.67, 127.70, 128.1, 128.4, 129.4 (2C), 130.9, 132.6, 133.5, 150.7, 170.0. *Anal.* Calcd for $C_{18}H_{14}O_2$: C, 82.4; H, 5.4. Found: C, 82.1; H, 5.3.

4-Chlorocarbonyl Benzaldehyde (16)[49] 4-Formylbenzoic acid (400 mg, 2.7 mmol) was dissolved in $SOCl_2$ (5 ml) and refluxed overnight. After evaporation of $SOCl_2$ the product was washed twice in CH_2Cl_2 and concentrated *in vacuo* to give the acid chloride in quantitative yield: mp 37—39 °C (lit.[49] 48 °C).

2-Naphthyl 4-Formylbenzoate (17) Compound **17** was prepared from **16** (134 mg, 0.79 mmol), 2-naphthol (115 mg, 0.80 mmol), triethylamine (0.12 ml, 0.87 mmol) and tetrahydrofuran (THF) (1 ml) as described above for the synthesis of **2**. The work-up was conducted as described for the preparation of **5**. The residue was purified by flash chromatog-

raphy [diethyl ether : petroleum ether (1 : 5) and CH_2Cl_2, respectively] to give 190 mg (86%) of the pure acid, mp 124—125 °C. IR (KBr) cm^{-1}: 1734 (ester), 1690 (aldehyde). ^{1}H-NMR ($CDCl_3$) δ: 7.36 (dd, J = 8.91, 1.98 Hz, 1H), 7.47—7.55 (m, 2H), 7.71 (d, J = 1.98 Hz, 1H), 7.82—8.41 (m, 4H), 7.92 (d, J = 8.91 Hz), 10.14 (s, 1H, CHO). ^{13}C-NMR ($CDCl_3$) δ: 118.6, 120.9, 126.0, 126.8, 127.7, 127.8, 129.7 (3C), 130.8 (2C), 131.6, 133.7, 134.5, 139.6, 148.3, 164.4, 191.6. *Anal.* Calcd for $C_{18}H_{12}O_3$: C, 78.3; H, 4.4. Found: C, 78.4; H, 4.5.

4-(2-Naphthyloxycarbonyl)benzoic Acid (7) Compound **7** was prepared according to a method described by Lindgren and Nilsson.[10] A solution of $NaClO_2$ (383 mg, 4.2 mmol) and $NaH_2PO_4 \times 1H_2O$ (454 mg, 3.3 mmol) in water (4.5 ml) was added dropwise to a mixture of **17** (130 mg, 0.47 mmol), *tert*-butanol (9.4 ml) and 2-methyl-2-butene (2.4 ml). The aqueous layer was acidified to pH 3. A solution of the crude product in methanol containing suspended silica gel was evaporated *in vacuo*. The silica plug was applied to a silica column, previously packed with diethyl ether. Flash chromatography [$CHCl_3$: methanol (9 : 1)] yielded 120 mg (87%) of a white powder, mp 260—261 °C. IR (KBr) cm^{-1}: 1730 (ester), 1685 (carboxylic acid). ^{1}H-NMR (DMSO-d_6) δ: 7.47—8.22 (m, 11H). ^{13}C-NMR (DMSO-d_6) δ: 118.7, 121.6, 126.0, 126.8, 127.6, 127.8, 128.7, 129.5, 129.6, 129.7, 130.8, 131.1, 131.6, 133.4, 140.6, 148.3, 164.6, 168.9. *Anal.* Calcd for $C_{18}H_{12}O_4$: C, 74.0; H, 4.1. Found: C, 74.0; H, 4.2.

4-Formylphenyl 2-Naphthoate (18) Compound **18** was synthesised from 2-naphthoyl chloride (260 mg, 1.4 mmol), 4-hydroxybenzaldehyde (200 mg, 1.6 mmol) and triethylamine (0.23 ml, 1.7 mmol) as described above for the synthesis of **2**. Partitioning between CH_2Cl_2 and water was followed by washing of the organic phase with water and aqueous saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by flash chromatography [$CHCl_3$: methanol (99 : 1)] provided 257 mg (68%) of the title compound, mp 119—119.5 °C. IR (KBr) cm^{-1}: 1733 (ester), 1705 (aldehyde). ^{1}H-NMR ($CDCl_3$) δ: 7.46—8.03 (m, 10H), 8.19 (dd, J = 8.58, 1.98 Hz, 1H), 8.80 (app s, 1H), 10.05 (s, 1H, CHO). ^{13}C-NMR ($CDCl_3$) δ: 122.6 (2C), 125.3, 126.0, 127.0, 127.9, 128.6, 128.9, 129.5, 131.3, 132.1, 132.3, 132.4, 134.1, 135.9, 155.8, 164.6, 190.9. *Anal.* Calcd for $C_{18}H_{12}O_3 \times 0.25H_2O$: C, 77.0; H, 4.5. Found C, 76.7; H, 4.3.

4-(2-Naphthoyloxy)benzoic Acid (8) Compound **8** was synthesised from **18** (209 mg, 0.76 mmol) as described above for the synthesis of **7**. The reaction mixture was stirred at room temperature overnight, diluted with $CHCl_3$ and extracted with aqueous saturated $NaHCO_3$. The aqueous layer was separated, acidified with 1 M HCl and extracted with $CHCl_3$. The organic phase was dried ($MgSO_4$), filtered and evaporated under reduced pressure providing 211 mg (95%) of **8**, mp 224—225 °C. IR (KBr) cm^{-1}: 1734 (ester), 1700 (carboxylic acid). ^{1}H-NMR (DMSO-d_6) δ: 7.48—8.24 (m, 10H), 8.88 (app s, 1H), 13.03 (br s, 1H, COOH). ^{13}C-NMR (DMSO-d_6) δ: 122.2 (2C), 125.0, 125.8, 127.2, 127.8, 128.6, 128.7, 129.1, 129.5, 131.0 (2C), 131.7, 132.1, 135.4, 154.2, 164.4, 166.6. *Anal.* Calcd for $C_{18}H_{12}O_4$: C, 74.0; H, 4.1. Found: C, 73.8; H, 4.2.

2-Naphthalenemethyl 4-Formylbenzoate (19) Compound **19** was prepared from **16** (100 mg, 0.59 mmol), 2-naphthalenemethanol (113 mg, 0.71 mmol), triethylamine (0.10 ml, 0.72 mmol) and a few drops of THF as described above for the synthesis of **2**. The work-up was conducted as described for compound **18**. The residue was purified by flash chromatography [diethyl ether : petroleum ether (1 : 3)], affording 120 mg (70%) of the title compound, mp 105—106 °C. IR (KBr) cm^{-1}: 1710 (ester). ^{1}H-NMR ($CDCl_3$) δ: 5.56 (s, 2H), 7.49—8.26 (m, 11H), 10.10 (s, 1H, CHO). ^{13}C-NMR ($CDCl_3$) δ: 67.5, 125.9, 126.4 (2C), 127.6, 127.7, 128.0, 128.5, 129.5 (2C), 130.3 (2C), 132.9, 133.15, 133.19, 135.0, 139.2, 165.4, 191.5. *Anal.* Calcd for $C_{19}H_{14}O_3$: C, 78.6; H, 4.9. Found: C, 78.5; H, 5.0.

4-(2-Naphthalenemethyloxycarbonyl)benzoic Acid (9) Compound **9** was prepared from **19** as described above for the synthesis of **7**. The yellow mixture was partitioned between $CHCl_3$ and water after having been stirred at room temperature for 2 h. Drying ($MgSO_4$), filtration and concentration *in vacuo* gave a white solid. Purification by flash chromatography [$CHCl_3$: methanol (9 : 1)] yielded 23 mg (91%) of **9** as a white powder, mp 225—226 °C. IR (KBr) cm^{-1}: 1719 (ester), 1681 (carboxylic acid). ^{1}H-NMR (DMSO-d_6) δ: 5.55 (s, 2H), 7.51—8.14 (m, 11H), 13.35 (br s, 1H, COOH). ^{13}C-NMR (DMSO-d_6) δ: 66.8, 126.0, 126.35, 126.41, 127.0, 127.6, 127.9, 128.2, 129.4, 129.5, 129.6, 132.66, 132.72, 133.1, 133.4, 134.5, 135.0, 165.0, 166.6. *Anal.* Calcd for $C_{19}H_{14}O_4$: C, 74.5; H, 4.6. Found: C, 74.5; H, 4.6.

4-Formylphenyl 2-Naphthylacetate (20) Compound **20** was prepared as described for the synthesis of **2**, using 2-naphthylacetyl chloride (279 mg, 1.36 mmol), 4-hydroxybenzaldehyde (200 mg, 1.64 mmol) and triethylamine (0.23 ml, 1.66 mmol). Work-up as described above for **19**, yielded 310 mg (78%) of the title compound, mp 71—72 °C. IR (KBr) cm^{-1}: 1753 (ester), 1703 (aldehyde). ^{1}H-NMR ($CDCl_3$) δ: 4.05 (s, 2H), 7.23—7.90 (m, 11H), 9.97 (s, 1H, CHO). ^{13}C-NMR ($CDCl_3$) δ: 41.5, 122.2 (2C), 126.1, 126.3, 127.1, 127.7 (2C), 128.16, 128.23, 128.5, 130.3, 131.1, 132.6, 133.4, 134.0, 155.3, 169.3, 190.8. *Anal.* Calcd for $C_{19}H_{14}O_3$: C, 78.6; H, 4.9. Found: C, 78.6; H, 4.8.

4-(2-Naphthylacetyloxy)benzoic Acid (12) Compound **12** was prepared from **20** (280 mg, 0.96 mmol) as described above for the synthesis of **7**. The reaction mixture was stirred at room temperature overnight, diluted with $CHCl_3$ and extracted with aqueous saturated $NaHCO_3$. The aqueous layer was separated, acidified with 1 M HCl and extracted with $CHCl_3$. The organic phase was dried ($MgSO_4$), filtered and evaporated under reduced pressure, providing 265 mg (90%) of the pure acid, mp 204—205 °C. IR (KBr) cm^{-1}: 1750 (ester), 1689 (carboxylic acid). ^{1}H-NMR (DMSO-d_6) δ: 4.19 (s, 2H), 7.27—8.01 (m, 11H), 12.98 (br s, 1H, COOH). ^{13}C-NMR (DMSO-d_6) δ: 40.3, 122.0 (2C), 125.9, 126.3, 127.5 (2C), 127.8, 128.0, 128.1, 128.4, 131.0 (2C), 131.4, 132.0, 133.0, 154.0, 166.6, 169.8. *Anal.* Calcd for $C_{19}H_{14}O_4$: C, 74.5; H, 4.6. Found: C, 74.5; H, 4.6.

Methyl 4-[[(Trifluoromethyl)sulfonoyl]oxy]phenylacetate (21) A solution of methyl (4-hydroxy)phenylacetate (1.0 g, 6.0 mmol) and pyridine (2.4 ml, 30 mmol) in CH_2Cl_2 (9 ml) was cooled in an ice bath. Trifluoromethanesulfonic anhydride (1.1 ml, 6.7 mmol) was added slowly. The reaction mixture was stirred for 45 min, diluted with water (20 ml) and partitioned between CH_2Cl_2 (30 ml) and 0.5 M NaOH (15 ml). The organic layer was washed successively with water (20 ml), 10% aqueous citric acid (2 × 25 ml) and water (20 ml). Drying ($MgSO_4$), filtration and concentration under reduced pressure yielded an oil, which was purified by flash chromatography [diethyl ether : petroleum ether (1 : 2)], providing 1.67 g (94%) of **21** as a crystalline product, mp 31—33 °C. IR (KBr) cm^{-1}: 1740 (ester). ^{1}H-NMR ($CDCl_3$) δ: 3.59 (s, 2H), 3.65 (s, 3H), 7.15—7.32 (m, 4H). ^{13}C-NMR ($CDCl_3$) δ: 40.3, 52.2, 118.7 (q, $J_{C,F}$ = 321 Hz, CF_3), 121.4 (2C), 131.2 (2C), 134.5, 148.6, 171.2. *Anal.* Calcd for $C_{10}H_9F_3O_5S$: C, 40.3; H, 3.0. Found: C, 40.4; H, 3.0.

4-[[(Trifluoromethyl)sulfonoyl]oxy]phenylacetic Acid (22) A mixture of **21** (384 mg, 1.29 mmol), TFA (10 ml, 0.13 mol) and one drop of 5 M NaOH was stirred under reflux at 72 °C for 9 h. The cooled mixture was partitioned between CH_2Cl_2 and water. Drying of the organic layer ($MgSO_4$), filtration and concentration *in vacuo* provided 338 mg (92%) of the acid as crystals, mp 81—82.5 °C. IR (KBr) cm^{-1}: 1700 (carboxylic acid). ^{1}H-NMR ($CDCl_3$) δ: 3.69 (s, 2H), 7.23—7.39 (m, 4H), 10.35 (br s, 1H, COOH). ^{13}C-NMR ($CDCl_3$) δ: 40.2, 118.7 (q, $J_{C,F}$ = 321 Hz, CF_3), 121.6 (2C), 131.3 (2C), 133.7, 148.9, 177.1. *Anal.* Calcd for $C_9H_7F_3O_5S$: C, 38.0; H, 2.5. Found: C, 38.0; H, 2.3.

Benzyl 4-[[(Trifluoromethyl)sulfonoyl]oxy]phenylacetate (23) Collected fractions of **22** (800 mg, 2.8 mmol) and powdered K_2CO_3 (389 mg, 2.8 mmol) were suspended in acetone (10 ml). Benzyl bromide (0.37 ml, 3.1 mmol) was added dropwise after 30 min. The reaction mixture was refluxed for 4 h and then partitioned between diethyl ether and water. The organic solution was washed with aqueous saturated $NaHCO_3$, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give an oil. Purification by flash chromatography [diethyl ether : petroleum ether (1 : 4)] yielded 1.0 g (95%) of a white solid, mp 40—41 °C. IR (KBr) cm^{-1}: 1734 (ester). ^{1}H-NMR ($CDCl_3$) δ: 3.69 (s, 2H, $-O(C=O)C\underline{H}_2Ar$), 5.14 (s, 2H, $PhC\underline{H}_2O(C=O)-$), 7.21—7.38 (m, 9H). ^{13}C-NMR ($CDCl_3$) δ: 40.5, 67.0, 118.7 (q, $J_{C,F}$ = 321 Hz, CF_3), 121.5 (2C), 128.2 (2C), 128.4, 128.6 (2C), 131.3 (2C), 134.5, 135.5, 148.7, 170.6. *Anal.* Calcd for $C_{16}H_{13}F_3O_5S$: C, 51.3; H, 3.5. Found: C, 51.4; H, 3.5.

4-[(Benzyloxycarbonyl)methyl] [2-(Trimethylsilyl)ethyl]benzoate (24) Carbon monoxide (CO) (toxic!) was bubbled through a solution of palladium(II)acetate [$Pd(OAc)_2$] (5.4 mg, 24 μmol) and 1,3-bis(diphenylphosphino)propane (dppp) (9.9 mg, 24 μmol) in dry dimethylformamide (DMF) (3 ml). A mixture of **23** (300 mg, 0.80 mmol), 2-(trimethylsilyl)ethanol (2 ml) and triethylamine (0.12 ml, 0.87 mmol), saturated with carbon monoxide, was added after 15 min. The reaction mixture was kept under an atmosphere of CO at 80 °C overnight. GC-MS analysis of the reaction mixture revealed remaining starting material. The reaction was therefore continued for 24 h after addition of a second portion of catalyst (5.4 mg) and bidentate ligand (9.9 mg). DMF was evaporated and the residue was dissolved in diethyl ether and extracted

with water. The organic layer was washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification of the crude ester by flash chromatography [diethyl ether : petroleum ether (1 : 4)] gave an oil, 233 mg (79%). IR (film) cm^{-1}: 1734 and 1714 (ester). ^{1}H-NMR ($CDCl_3$) δ: 0.09 (s, 9H), 1.10—1.16 (m, 2H, $-OCH_2C\underline{H}_2TMS$), 3.72 (s, 2H, $-O(C=O)C\underline{H}_2Ph$), 4.39 —4.45 (m, 2H, $-OC\underline{H}_2CH_2TMS$), 5.14 (s, 2H, $PhC\underline{H}_2O-$), 7.32—8.01 (m, 9H). ^{13}C-NMR ($CDCl_3$) δ: −1.4 (3C). 17.4, 41.3, 63.2, 66.8, 128.2 (2C), 128.3, 128.6 (2C), 129.3 (2C), 129.6, 129.7 (2C), 135.7, 138.8, 166.5, 170.7. *Anal.* Calcd for $C_{21}H_{26}O_4Si$: C, 68.1; H, 7.1. Found: C, 67.9; H, 7.0.

4-[2-(Trimethylsilyl)ethoxycarbonyl]phenylacetic Acid (25) Compound **24** (205 mg, 0.55 mmol) was dissolved in THF (15 ml) and hydrogenated over 10% Pd/C under a balloon for 3 h. Filtration through a pad of Celite and concentration *in vacuo* afforded 129 mg (83%) of the pure acid, mp 63—65 °C. IR (KBr) cm^{-1}: 1718 (ester), 1701 (carboxylic acid). ^{1}H-NMR ($CDCl_3$) δ: 0.08 (s, 9H), 1.09—1.15 (m, 2H, $-OCH_2C\underline{H}_2TMS$), 3.68 (s, 2H), 4.37—4.44 (m, 2H, $-OC\underline{H}_2CH_2TMS$), 7.32—8.01 (m, 4H), 9.35 (br s, 1H, COOH). ^{13}C-NMR ($CDCl_3$) δ: −1.4 (3C), 17.4, 41.0, 63.4, 129.5 (2C), 129.72, 129.8 (2C), 138.3, 166.6, 177.0. *Anal.* Calcd for $C_{14}H_{20}O_4Si$: C, 59.9; H, 7.2. Found: C, 59.9; H, 7.1.

4-[(Chlorocarbonyl)methyl] [2-(Trimethylsilyl)ethyl]benzoate (26) Oxalyl chloride (62 μl, 0.71 mmol) was added to a solution of **25** (100 mg, 0.36 mmol) in toluene (5 ml). The mixture was heated on a water bath at about 40 °C until the production of bubbles ceased. The solvent was evaporated under reduced pressure, giving the acid chloride in quantitative yield.

4-[(2-Naphthyloxycarbonyl)methyl] [2-(Trimethylsilyl)ethyl]benzoate (27) Compound **27** was prepared from **26** (105 mg, 0.35 mmol), 2-naphthol (54 mg, 0.37 mmol) and triethylamine (54 μl, 0.39 mmol) as described above for the synthesis of **2**. Work-up was performed as described for **2**. Purification of the residue by flash chromatography [diethyl ether : petroleum ether (1 : 4)], followed by preparative TLC (CH_2Cl_2) provided 89 mg (62%) of **27** as a crystalline product, mp 68—70 °C. IR (KBr) cm^{-1}: 1752 and 1718 (ester). ^{1}H-NMR ($CDCl_3$) δ: 0.09 (s, 9H), 1.12—1.18 (m, 2H, $-OCH_2C\underline{H}_2TMS$), 3.98 (s, 2H), 4.40—4.47 (m, 2H, $-OCH_2CH_2TMS$), 7.17—8.08 (m, 11H). ^{13}C-NMR ($CDCl_3$) δ: −1.5 (3C), 17.4, 41.4, 63.3, 118.4, 120.8, 125.8, 126.6, 127.6, 127.7, 129.38 (2C), 129.42, 129.9 (2C), 131.5, 133.7, 138.3, 148.2, 166.4, 169.4. *Anal.* Calcd for $C_{24}H_{26}O_4Si$: C, 70.9; H, 6.4. Found: C, 70.8; H, 6.6.

4-[(2-Naphthyloxycarbonyl)methyl]benzoic Acid (10) Trimethylsilyl chloride (0.16 ml, 1.3 mmol) was added *via* a dry syringe to a mixture of **27** (260 mg, 0.64 mmol) and sodium iodide (192 mg, 1.3 mmol) in acetonitrile (5 ml). The reaction mixture was refluxed for 1 h, then partitioned between $CHCl_3$ and water. The organic solution was washed with 10% aqueous $Na_2S_2O_3$, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude acid was taken up in $CHCl_3$ and extracted with aqueous saturated Na_2CO_3. The aqueous phase was separated, acidified with 5 M HCl and extracted with $CHCl_3$. Drying ($MgSO_4$) of the organic layer, filtration and concentration under reduced pressure provided 184 mg (94%) of **10** as a white powder, mp 189—190 °C. IR (KBr) cm^{-1}: 1752 (ester), 1702 (carboxylic acid). ^{1}H-NMR (DMF-d_6) δ: 4.21 (s, 2H), 7.38 (dd, J=8.91, 2.31 Hz, 1H), 7.51—7.60 (m, 4H), 7.74 (d, J=2.31 Hz, 1H), 7.95—8.08 (m, 5H), 13.20 (br s, 1H, COOH). ^{13}C-NMR (DMF-d_6) δ: 40.9, 119.2, 122.1, 126.5, 127.4, 128.2, 128.4, 130.1, 130.3 (2C), 130.5 (2C), 130.7, 132.1, 134.4, 140.0, 149.3, 167.9, 170.7. *Anal.* Calcd for $C_{19}H_{14}O_4 \times 0.25H_2O$: C, 73.4; H, 4.7. Found: C, 73.2; H, 5.0.

[2-(Trimethylsilyl)ethyl] 4-Bromomethylbenzoate (28) 4-Bromomethylbenzoic acid (200 mg, 0.93 mmol), 2-(trimethylsilyl)ethanol (0.27 ml, 1.9 mmol), *N,N'*-dicyclohexylcarbodiimide (384 mg, 1.9 mmol) and a catalytic amount of 4-dimethylaminopyridine were dissolved in CH_2Cl_2 (5 ml). The reaction mixture was stirred at room temperature for 18 h. The precipitate was filtered off and the filtrate was partitioned between CH_2Cl_2 and aqueous saturated $NaHCO_3$. The organic layer was washed with 0.5 M HCl and water. Drying ($MgSO_4$), filtration and evaporation *in vacuo*, followed by purification by flash chromatography [ethyl acetate : pentane (1 : 9)], gave 268 mg (91%) of a colourless oil. IR (diethyl ether) cm^{-1}: 1708 (ester). ^{1}H-NMR ($CDCl_3$) δ: 0.08 (s, 9H), 1.10—1.16 (m, 2H, $-OCH_2C\underline{H}_2TMS$), 4.39—4.45 (m, 2H, $-OC\underline{H}_2CH_2TMS$), 4.50 (s, 2H), 7.43—8.02 (m, 4H). ^{13}C-NMR ($CDCl_3$) δ: −1.5 (3C), 17.4, 32.3, 63.4, 128.4, 129.0 (2C), 130.0 (2C), 130.6, 166.1. HR-MS Calcd for $C_{13}H_{19}BrO_2Si + C_2H_9N_2$ (1,2-diaminoethane): 375.1103. Found: 375.1112 $(M+H)^+$.

4-[(2-Naphthoyloxy)methyl] [2-(Trimethylsilyl)ethyl]benzoate (29) 2-Naphthoic acid (126 mg, 0.73 mmol) and powdered K_2CO_3 (101 mg, 0.73 mmol) were suspended in acetone (2.5 ml). Compound **28** (210 mg, 0.67 mmol) was added 30 min later and the reaction mixture was stirred at room temperature for 4 d. The crude mixture was partitioned between diethyl ether and water. The ether layer was dried ($MgSO_4$) and concentrated *in vacuo* to afford an oil, which was purified by flash chromatography [diethyl ether : pentane (1 : 5)] to give 262 mg (97%) of **29**, mp 70.5—71.5 °C. IR (diethyl ether) cm^{-1}: 1722 (ester). ^{1}H-NMR ($CDCl_3$) δ: 0.09 (s, 9H), 1.11—1.14 (m, 2H, $-OCH_2C\underline{H}_2TMS$), 4.40—4.46 (m, 2H, $-OC\underline{H}_2CH_2TMS$), 5.48 (s, 2H), 7.54—8.09 (m, 10H), 8.65 (app s, 1H). ^{13}C-NMR ($CDCl_3$) δ: −1.5 (3C), 17.4, 63.3, 66.0, 125.2, 126.7, 127.0, 127.7 (3C), 127.8, 128.2, 128.4, 129.3, 129.8 (2C), 130.5, 131.3, 132.4, 135.6, 140.9, 166.4. *Anal.* Calcd for $C_{24}H_{26}O_4Si$: C, 70.9; H, 6.4. Found: C, 70.7; H, 6.3.

4-[(2-Naphthoyloxy)methyl]benzoic Acid (11) TBAF, 1 M in THF (1.9 ml, 1.9 mmol), was added to a solution of **29** (195 mg, 0.48 mmol) in THF (4 ml) and the reaction mixture was stirred at room temperature for 30 min, then concentrated. The resulting oil was dissolved in $CHCl_3$ and the solution was extracted with aqueous saturated $NaHCO_3$. The aqueous phase was separated, acidified with 2 M HCl and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$), filtered, concentrated *in vacuo* and finally held under a vacuum pump (60 °C), affording 105 mg (71%) of the pure acid, mp 199—201 °C. IR (KBr) cm^{-1}: 1720 (ester), 1680 (carboxylic acid). ^{1}H-NMR (DMSO-d_6) δ: 5.51 (s, 2H), 7.61—8.19 (m, 10H), 8.71 (app s, 1H), 13.00 (br s, 1H, COOH). ^{13}C-NMR (DMSO-d_6) δ: 65.7, 124.8, 126.7, 127.0, 127.7 (3C), 128.5, 128.7, 129.4, 129.5 (2C), 130.6, 130.7, 132.1, 135.1, 141.0, 165.6, 167.0. *Anal.* Calcd for $C_{19}H_{14}O_4 \times 1H_2O$: C, 70.4; H, 5.0. Found: C, 70.2; H, 4.9.

2,4,6-Triaminoquinazoline (30)[19,20] A mixture of 2,4-diamino-6-nitroquinazoline (10.0 g, 48.8 mmol), DMF (80 ml) and acetic acid (0.8 ml) was hydrogenated over 10% Pd/C (1.0 g) under 60 psi for 3 h, filtered through Celite and concentrated *in vacuo*. Ethyl acetate was poured over the oily residue under stirring and the precipitate was filtered off to give a yellow/green solid, which showed a single spot on TLC. The crude compound was used in the next step without further purification. ^{1}H-NMR (DMSO-d_6) δ: 4.76 (br s, 2H, 6-NH_2), 5.49 (br s, 2H, 2-NH_2), 6.87 (br s, 2H, 4-NH_2), 6.95—7.03 (m, 3H). ^{13}C-NMR (DMSO-d_6) δ: 104.5, 110.4, 120.8, 123.7, 137.5, 143.8, 156.1, 162.0.

2,4-Diaminoquinazoline-6-carbonitrile (31)[19,20] A cold solution of $NaNO_2$ (1.2 g, 17.4 mmol) in water (9 ml) was added to a precooled (0 °C) solution of 2 M HCl (35 ml) and **30** (3.0 g, 17.1 mmol). The mixture was stirred until it was totally clear and then added to a warm (55 °C) mixture of $Cu(II)SO_4 \times 5H_2O$ (4.3 g, 17.2 mmol) in water (13 ml) and KCN (5.6 g, 86.0 mmol) in water (10 ml). The reaction mixture was thereafter stirred for 30 min at 55 °C and treated with concentrated aqueous ammonia (17 ml). Stirring was continued for 1 h at room temperature, then the precipitate was collected by filtration. The brown paste was added to boiling 15% AcOH (46 ml) and filtered hot under suction. The warm filtrate was diluted with 2-methoxyethanol (39 ml) and aqueous ammonia (14 ml) and the mixture was stored in the refrigerator overnight. Filtration and washing with cool water afforded 2.3 g (73%) of the nitrile, which gave a single spot on TLC, mp >360 °C (lit.[19] >360 °C). IR (KBr) cm^{-1}: 2222 (nitrile). ^{1}H-NMR (DMSO-d_6) δ: 6.66 (br s, 2H, 2-NH_2), 7.30 (d, J=8.58 Hz, 1H, H-8), 7.68 (br s, 2H, 4-NH_2), 7.81 (app d, J=8.57 Hz, 1H, H-7), 8.60 (app s, 1H, H-5). ^{13}C-NMR (DMSO-d_6) δ: 100.6, 110.1, 119.6, 125.2, 130.1, 133.7, 155.3, 162.1, 162.5.

2,4-Diaminoquinazoline-6-carbaldehyde (32)[19,20] A combination of a method described by van Es *et al.*[50] and a modified version described by Piper *et al.*[51] was adopted for the synthesis of the aldehyde **32**. Compound **31** (2.24 g, 12.1 mmol), Ni–Al alloy (4 g) and 75% HCO_2H were refluxed for 75 min and then filtered while hot and washed with boiling 75% HCO_2H until the washings were colourless. The filtrate was concentrated *in vacuo* and washed with ethanol. The aldehyde was recrystallised from hot water (230 ml) and boiled with a small portion of active charcoal. Filtration while hot through a pad of Celite and precipitation of the base with 1 M NaOH gave, after storage in the refrigerator overnight and collection by filtration under suction, 2.12 g (93%) of a beige solid, mp >360 °C (lit.[19] >360 °C). IR (KBr) cm^{-1}: 2825, 1661 (aldehyde). ^{1}H-NMR (DMSO-d_6) δ: 6.82 (br s, 2H, 2-NH_2), 7.27 (d, J=8.58 Hz, 1H, H-8), 7.78 (br s, 2H, 4-NH_2), 7.93 (dd, J=8.58, 1.65 Hz, 1H, H-7), 8.63 (d, J=1.65 Hz, 1H, H-5), 9.85 (s, 1H, CHO). ^{13}C-NMR (DMSO-d_6) δ: 109.6, 124.3, 128.5, 129.7, 130.7, 156.2, 162.1, 163.1, 190.4.

2,4-Bis(pivaloylamino)quinazoline-6-carbaldehyde (33) Compound **32**

(80 mg, 0.42 mmol) was heated to 80 °C together with pivalic anhydride (0.19 ml, 0.94 mmol) in DMF (1 ml). TLC indicated remaining starting material after 8 h. The reaction was continued for 60 hours until all the amine had been consumed. Concentration under reduced pressure was followed by purification by flash chromatography [ethyl acetate : pentane (1 : 4)]. The white powder was dissolved in ethyl acetate and washed with aqueous saturated $NaHCO_3$. Drying ($MgSO_4$) and filtration afforded 125 mg (82%) of **33** as a white solid, mp 216—217 °C. IR (KBr) cm^{-1}: 3435 (amide), 2872, 1696 (aldehyde). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (s, 9H), 1.38 (s, 9H), 7.45 (d, J=8.58 Hz, 1H, H-8), 8.14 (dd, J=8.57, 1.98 Hz, 2H, NH, H-7), 8.89 (d, J=1.98 Hz, 1H, H-5), 10.05 (s, 1H, CHO), 15.64 (br s, 1H, NH). ^{13}C-NMR ($CDCl_3$) δ: 27.1 (3C), 27.6 (3C), 40.4, 42.7, 119.7, 126.7, 131.4, 132.8, 133.3, 146.5, 152.7, 154.9, 179.3, 190.8, 194.9. *Anal.* Calcd for $C_{19}H_{24}N_4O_3$: C, 64.0; H, 6.8; N, 15.7. Found: C, 64.1; H, 6.7; N, 15.6.

2,4-Bis(pivaloylamino)quinazoline-6-carboxylic Acid (34) Compound **34** was prepared from pooled fractions of **33** (500 mg, 1.4 mmol) as described above for the synthesis of **7**. The mixture was partitioned between $CHCl_3$ and water after having been stirred at room temperature for 90 min. The organic phase was dried ($MgSO_4$), filtered and evaporated *in vacuo*. The residue was partitioned between aqueous saturated $NaHCO_3$ and $CHCl_3$. The aqueous phase was separated, acidified with 2 M HCl and extracted with $CHCl_3$. Drying ($MgSO_4$) and concentration under reduced pressure was followed by drying under a vacuum pump to give 510 mg (98%) of a white powder, mp >360 °C (decomp.). IR (KBr) cm^{-1}: 3442 (amide), 1709 (carboxylic acid). ^{1}H-NMR (DMSO-d_6) δ: 1.32 (s, 9H), 1.39 (s, 9H), 7.59 (d, J=8.58 Hz, 1H, H-8), 8.34 (dd, J=8.58, 1.98 Hz, 1H, H-7), 9.14 (app s, 1H, H-5), 11.20 (br s, 1H, NH), 13.21 (br s, 1H, COOH), 15.40 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 26.7 (3C), 27.8 (3C), 40.9, 42.6, 119.4, 126.9, 127.7, 128.8, 135.4, 148.0, 152.4, 155.8, 167.2, 181.4, 193.4. *Anal.* Calcd for $C_{19}H_{24}N_4O_4 \times 0.5H_2O$: C, 59.6; H, 6.2; N, 14.6. Found: C, 59.8; H, 6.6; N, 14.7.

2,4-Bis(pivaloylamino)quinazoline-6-carboxylic Acid Chloride (35) Compound **35** was prepared from **34** (145 mg, 0.39 mmol) as described above for the synthesis of **25**. The reaction was interrupted after 90 min and the solvent was evaporated, giving the acid chloride in quantitative yield.

Benzyl 2,4-Bis(pivaloylamino)quinazoline-6-carboxylate (36) A solution of **35** (152 mg, 0.39 mmol) in CH_2Cl_2 (4 ml) was added dropwise to a precooled mixture (0 °C) of benzyl alcohol (44 μl, 0.43 mmol) and triethylamine (59 μl, 0.43 mmol) in CH_2Cl_2 (3 ml). The reaction mixture was allowed to reach room temperature overnight. Partition between CH_2Cl_2 and water, then drying ($MgSO_4$), filtration and evaporation of the organic solution under reduced pressure yielded, after purification by flash chromatography [ethyl acetate : pentane (1 : 4)], 100 mg (56%) of the pure ester, mp 221—222 °C. IR (KBr) cm^{-1}: 3440 (amide), 1709 (ester). ^{1}H-NMR ($CDCl_3$) δ: 1.35 (s, 9H), 1.36 (s, 9H), 5.42 (s, 2H), 7.32—7.51 (m, 6H), 8.14 (br s, 1H, NH), 8.33 (dd, J=1.98, 8.58 Hz, 1H, H-7), 9.24 (d, J=1.65 Hz, 1H, H-5), 15.43 (br s, 1H, NH). ^{13}C-NMR ($CDCl_3$) δ: 27.1 (3C), 27.6 (3C), 40.3, 42.6, 66.8, 119.4, 125.7, 127.0, 127.9 (2C), 128.2, 128.6 (2C), 129.3, 134.9, 135.9, 146.1, 151.4, 155.0, 165.4, 179.2, 194.8. *Anal.* Calcd for $C_{26}H_{30}N_4O_4 \times 0.5H_2O$: C, 66.2; H, 6.6; N, 11.9. Found: C, 66.3; H, 6.3; N, 11.9.

Benzyl 2,4-Diaminoquinazoline-6-carboxylate (13) Compound **36** was deprotected using a method described by Taylor *et al.*[24] Aqueous ammonia (6.5 ml) was added to collected fractions of **36** (130 mg, 0.28 mmol) dissolved in dioxane (13 ml) and the reaction mixture was stirred at room temperature for 8 d. The solvent was evaporated *in vacuo* and the solid was evaporated on silica gel, dispersed in methanol. The silica gel plug was put on the top of a silica column previously packed with CH_2Cl_2. The compound was purified by flash chromatography using $CHCl_3$: methanol (19 : 1) as the eluent. The reaction was not complete and the remaining monoprotected material (75 mg) was pooled and dissolved in dioxane (7 ml) and aqueous ammonia (3.5 ml). This solution was stirred for an additional 7 d. Purification was conducted as before and the collected material was recrystallised from methanol, furnishing 53 mg (64%) of **13**, mp 239—241 °C. IR (KBr) cm^{-1}: 3439 and 3339 (amine), 1710 (ester). ^{1}H-NMR (DMF-d_6) δ: 5.41 (s, 2H), 6.75 (br s, 2H, 2-NH_2), 7.33 (d, J=8.81 Hz, 1H, H-8), 7.37—7.55 (m, 5H), 8.03 (br s, 2H, 4-NH_2), 8.11 (dd, J=8.81, 1.92 Hz, 1H, H-7), 8.93 (d, J=1.92 Hz, 1H, H-5). ^{13}C-NMR (DMF-d_6) δ: 66.7, 110.6, 122.3, 124.3, 127.7, 128.6 (2C), 128.7, 129.2 (2C), 133.3, 137.5, 155.2, 162.6, 164.4, 166.3. *Anal.* Calcd for $C_{16}H_{14}N_4O_2 \times 0.5H_2O$: C, 63.6; H, 5.2; N, 18.4. Found: C, 63.4; H, 5.0; N, 18.5.

2,4-Bis(pivaloylamino)quinazoline-6-methanol (37) Small portions of $NaBH_4$ (64 mg, 1.7 mmol) were added to a mixture of pooled fractions of **33** (600 mg, 1.7 mmol) in methanol (40 ml). The reaction mixture was stirred at room temperature for 30 min. The solvent was evaporated and the residue was partitioned between ethyl acetate and saturated aqueous NH_4Cl. Drying ($MgSO_4$), filtration and concentration of the organic solution *in vacuo* was followed by purification by flash chromatography [$CHCl_3$: methanol (39 : 1) and (79 : 1), respectively], yielding 505 mg (84%) of the alcohol, mp 215—217 °C. IR (KBr) cm^{-1}: 3630—3096 (alcohol). ^{1}H-NMR ($CDCl_3$) δ: 1.34 (s, 9H), 1.36 (s, 9H), 5.91 (s, 2H), 7.39 (d, J=8.24 Hz, 1H, H-8), 7.71 (s, 1H), 7.74 (dd, J=1.98, 8.25 Hz, 1H, H-7), 8.16 (br s, 1H, NH), 8.44 (app s, 1H, H-5), 15.60 (br s, 1H, NH). ^{13}C-NMR ($CDCl_3$) δ: 27.1 (3C), 27.7 (3C), 40.3, 42.5, 64.5, 119.2, 124.5, 125.5, 134.2, 138.8, 144.5, 147.3, 155.2, 179.1, 194.8. *Anal.* Calcd for $C_{19}H_{26}N_4O_3 \times 0.25H_2O$: C, 62.9; H, 7.4; N, 15.4. Found: C, 63.2; H, 7.3; N, 15.2.

[2,4-Bis(pivaloylamino)quinazoline-6-methyl] Benzoate (38) Compound **38** was prepared from **37** (500 mg, 1.4 mmol) as described above for the synthesis of **36**. A TLC analysis revealed remaining starting material, so additional benzoyl chloride (0.1 eq) was added and the reaction was continued for 6 h. Partitioning between CH_2Cl_2 and aqueous saturated $NaHCO_3$, followed by washing of the organic solution with water, drying ($MgSO_4$), filtration and evaporation *in vacuo* yielded, after purification by flash chromatography [ethyl acetate : pentane (1 : 3)], 515 mg (80%) of the pure ester, mp 178—179 °C. IR (KBr) cm^{-1}: 1718 (ester). ^{1}H-NMR ($CDCl_3$) δ: 1.34 (s, 9H), 1.36 (s, 9H), 5.49 (s, 2H), 7.42—7.48 (m, 3H), 7.55—7.61 (m, 1H), 7.79 (dd, J=8.58, 1.98 Hz, 1H, H-7), 8.08 (br s, 1H, NH), 8.10—8.14 (m, 2H), 8.62 (d, J=1.98 Hz, 1H, H-5), 15.78 (br s, 1H, NH). ^{13}C-NMR ($CDCl_3$) δ: 26.9 (3C), 27.6 (3C), 40.1, 42.4, 65.7, 119.4, 125.4, 125.8, 128.3 (2C), 129.5 (2C), 129.7, 133.0, 133.5, 134.2, 144.6, 147.9, 155.1, 166.1, 179.0, 194.4. *Anal.* Calcd for $C_{26}H_{30}N_4O_4 \times 0.5H_2O$: C, 66.2; H, 6.6; N, 11.9. Found: C, 66.5; H, 6.4; N, 11.8.

[2,4-Diaminoquinazoline-6-methyl] Benzoate (14) Compound **38** was deprotected as described earlier for **13**, starting with 400 mg of **38**. The reaction mixture was stirred at room temperature for 3 d. The solid, collected by evaporation of the solvent *in vacuo*, was suspended in methanol together with a small portion of silica gel. The silica gel plug was applied to the top of a silica column previously packed with CH_2Cl_2, and the residue was purified by flash chromatography [$CHCl_3$: methanol (19 : 1)]. As in the case of **14**, remaining monoprotected compound was stirred for a few more days under the same conditions as before. Purification was performed as described above and the collected fractions were recrystallised from methanol to give 107 mg (42%) of the pure compound, mp 210—211 °C. IR (KBr) cm^{-1}: 1702 (ester). ^{1}H-NMR (DMF-d_6) δ: 5.40 (s, 2H), 6.25 (br s, 2H, 2-NH_2), 7.31 (d, J=8.58 Hz, 1H, H-8), 7.44 (br s, 2H, 4-NH_2), 7.52—7.71 (m, 4H), 8.03—8.08 (m, 2H), 8.24 (d, J=1.65 Hz, 1H, H-5). ^{13}C-NMR (DMF-d_6) δ: 67.4, 111.0, 124.7, 125.7, 128.5, 129.4 (2C), 130.0 (2C), 130.9, 133.7, 133.9, 153.8, 162.4, 163.8, 166.6. *Anal.* Calcd for $C_{16}H_{14}N_4O_2 \times 0.8H_2O$: C, 62.2; H, 5.1; N, 18.1. Found: C, 61.9; H, 4.7; N, 18.1.

2,4-Diaminoquinazoline-6-carboxylic Acid (39) Compound **39** was prepared from **32** (150 mg, 0.80 mmol), adopting the method described above for the synthesis of **7**. The reaction mixture was stirred overnight, after which the solvent was evaporated. The residue was alkalised with 1 M NaOH and filtered. Addition of 1 M HCl to make pH 4 gave a paste after filtration which was dissolved in a small portion of 0.5 M NaOH and purified on a cellulose column (Avicel) with 0.25% NH_4HCO_3.[52] The pooled fractions were washed with water, acetone and diethyl ether, yielding 50 mg (31%) of **39**, after drying at room temperature, as an off-white solid, mp >360 °C. IR (KBr) cm^{-1}: 1705 (carboxylic acid). ^{1}H-NMR (DMSO-d_6) δ: 6.57 (br s, 2H, 2-NH_2), 7.21 (d, J=8.58 Hz, 1H), 7.64 (br s, 2H, 4-NH_2), 8.04 (dd, J=8.57, 1.65 Hz, 1H), 8.68 (d, J=1.65 Hz, 1H). ^{13}C-NMR (DMSO-d_6 and one drop of CF_3COOD) δ: 109.1, 117.1, 126.5, 127.1, 135.4, 142.3, 154.9, 166.1, 163.0. HR-MS Calcd for $C_9H_8N_4O_2$: 204.0647. Found: 204.0648 (M^+).

2,4-Diaminoquinazoline-6-methanol (40)[53] Small portions of $NaBH_4$ (201 mg, 5.3 mmol) were added to a stirred solution of **32** (1.0 g, 5.3 mmol) in methanol (400 ml). Stirring was continued for 40 min at room temperature until TLC indicated full conversion to the alcohol. The mixture was filtered, water (10 ml) was added to the filtrate, and the aqueous phase was evaporated under reduced pressure to about 7 ml. The precipitate formed on storage in a refrigerator overnight, was

collected by filtration, affording 703 mg (70%) of the alcohol as a yellowish powder, mp 259—262 °C. IR (KBr) cm^{-1}: 3510—2980 (alcohol). ^{1}H-NMR (DMSO-d_6) δ: 4.48 (d, 2H, $ArC\underline{H}_2OH$), 5.15 (t, 1H, $CH_2O\underline{H}$), 5.92 (br s, 2H, 2-NH_2), 7.16 (d, J=8.58 Hz, 1H, H-8), 7.22 (br s, 2H, 4-NH_2), 7.47 (dd, J=8.58, 1.98 Hz, 1H, H-7), 7.88 (app s, 1H, H-5). ^{13}C-NMR (DMSO-d_6) δ: 63.1, 109.8, 121.4, 124.1, 131.9, 134.0, 151.7, 160.6, 162.5. *Anal.* Calcd for $C_9H_{10}N_4O \times 0.10H_2O$: C, 56.3; H, 5.4; N, 29.2. Found: C, 56.3; H, 5.4; N, 29.0.

Measuring Rates of Hydrolysis Incubation Procedure The pure enzymes and the biological matrices were diluted with buffer (50 mM K_2HPO_4 pH 7.5 with 0.05% EDTA) to a protein concentration yielding a measurable rate of hydrolysis. Incubation with sonicated human leukocytes was done at a cell concentration of 14×10^6 cells per ml. When cholesterol esterase was used, taurocholate was added to a final concentration of 10 mM. Prior to the addition of the substrates, the incubation solutions were kept at 37 °C in a gently shaken water bath for about three minutes. The substances were added at time zero as DMSO solutions, yielding a concentration of 50 μM. The amount of DMSO present during the incubations was 5% throughout all the experiments. The incubations were continued in a water bath at 37 °C and samples of 150 μl were withdrawn and immediately added to an aliquot of 100% CH_3CN, and put on ice to stop the enzymatic reaction. The samples were kept on ice for a few minutes, then centrifuged at 15000 *g* for 4 min to remove the protein fraction, injected into the LC system and analysed. The incubation time never exceeded 60 min.

Analysis The samples were chromatographed on a Supelco C_{18} column (3 μm, 15 cm × 4.6 mm) operated at a flow rate of 1 ml/min. The UV detector was set at 220 or 230 nm depending on the substances measured. Two mobile phases were used to create a gradient for elution of the substrates and the formed metabolites. Mobile phase A consisted of 50 mM H_3PO_4 pH 3.0 and mobile phase B contained 75% CH_3CN (v/v). The gradient was adjusted as required for different substances and corresponding bicyclic aromatic metabolites. The approximate duration of a chromatographic run was 20 min.

Rates of Hydrolysis The chromatographic system was optimised for the bicyclic aromatic metabolites formed. These bicyclic aromatic metabolites were quantified and their formation was used to determine the initial hydrolytic rates of the substrates. Commercially available PLE was obtained as an enzyme suspension (carboxyl esterase from porcine liver; Sigma E-3128). The unit for the rates of hydrolysis with PLE were expressed as nmol/ml/min and calculated to a dilution of the enzyme suspension of 1/8000, which was the highest enzyme concentration used. The cholesterol esterase was obtained as a lyophilised powder (bovine pancreas; Sigma C-3766). The unit of hydrolysis for this enzyme was expressed as nmol/ml/min/mg lyophilised enzyme powder. The hydrolyses in human duodenal mucosa and S9 fractions from human and rat liver were expressed as nmol/ml/min/mg protein. The calculations for the hydrolysis with human leukocytes were related to a cell concentration of 10^6 cells/ml. The unit for the hydrolysis was consequently expressed as nmol/ml/min/10^6 cells.

Human duodenal tissue was obtained from the Surgery Department, Lund University Hospital, Sweden, and human liver from the Liver Bank, Huddinge Hospital, Sweden. Blood for preparation of leukocytes was obtained from volunteers at our laboratory.

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