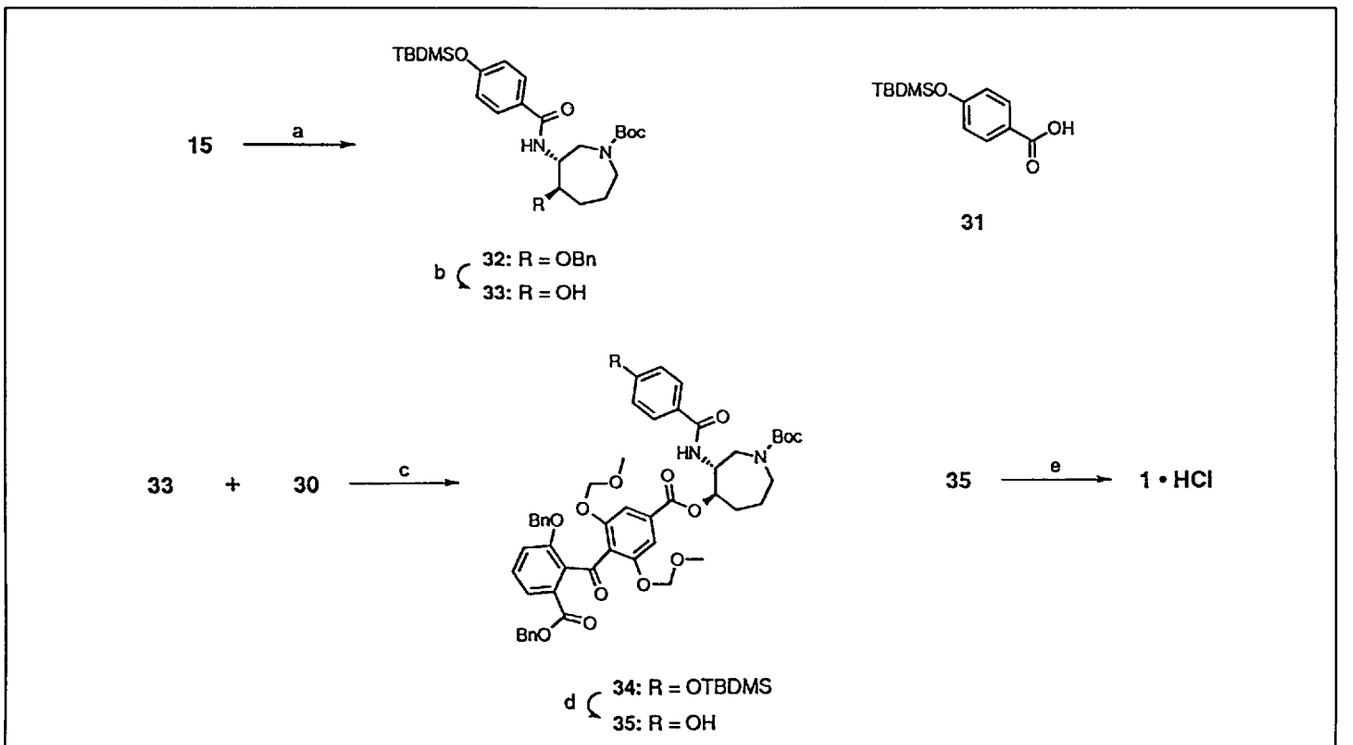
CHIMIA 50 (1996) Nr. 11 (November)

Scheme 3



a) 1. H_2, Pd/C, THF; 2. + **31**, *N*-[3-(dimethylamino)propyl]-*N'*-ethyl-carbodiimide hydrochloride (EDC), DMAP, CH_2Cl_2. b) H_2, Pd/C, MeOH. c) *p*-TsCl, DMAP, CH_2Cl_2. d) $(Bu)_4NF$, THF. e) 1. H_2, Pd-C, THF; 2. HCl (g), i-PrOH, DME.

[7] R.J. Ferrier, N. Prasad, *J. Chem. Soc. (C)* **1969**, 570.
[8] R.J. Ferrier, *Methods Carbohydr. Chem.* **1972**, *6*, 307.
[9] M. Menard, L. Erichomovitch, M. La Brooy, F.L. Chubb, *Can. J. Chem.* **1963**, *41*, 1722.
[10] L.L. Chang, J. Hannah, W.T. Ashton, G.H. Rasmusson, T.J. Ikeler, G.F. Patel, V. Garsky, C. Uncapher, G. Yamanaka, W.L. McClements, R.L. Tolman, *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1207.

Chimia 50 (1996) 532–537

ISSN 0009–4293

Synthesis of the HIV-Proteinase Inhibitor Saquinavir: A Challenge for Process Research

Wolfgang Göhring, Surendra Gokhale, Hans Hilpert*, Felix Roessler, Markus Schlageter and Peter Vogt

Abstract. The task of process research, namely developing efficient, economically and technically as well as ecologically feasible syntheses in time, is demonstrated on the HIV-proteinase inhibitor Saquinavir (**1**), a complex molecule comprising six stereocentres. Based on the first 26-step research synthesis furnishing a 10% overall yield, process research established a new, short 11-step synthesis affording a 50% overall yield.

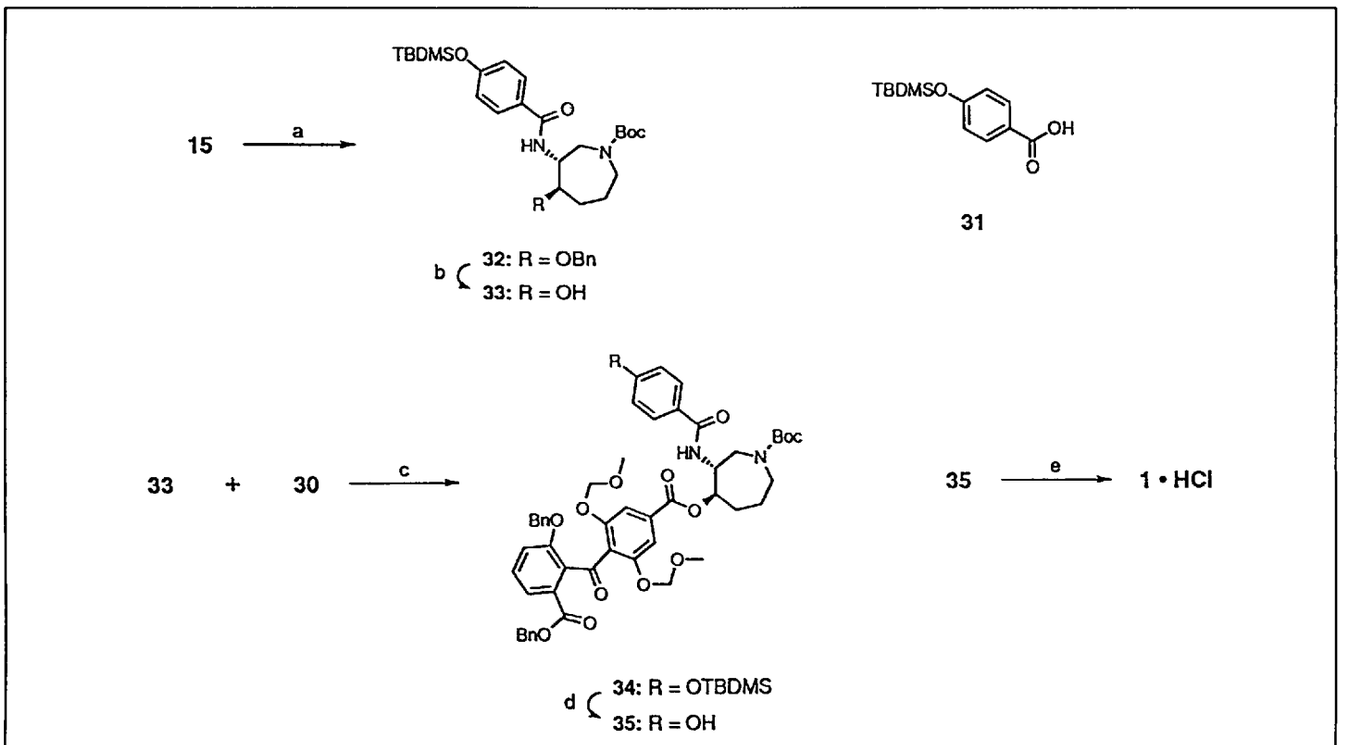
1. Introduction

In 1986, the HIV-proteinase, an enzyme that catalyses the processing of the group antigen (gag) polyprotein p55 to the core proteins p24, p17 and p15, was recognized by *Kramer* [1] as a challenging new target to combat acquired immunodeficiency syndrome (AIDS). Subsequently, industry as well as academia started an intensive search for inhibitors of the HIV-proteinase. At our research laboratories in Welwyn, England, a number of potent inhibitors were synthesized and structurally optimized leading finally to the selection of the peptide mimetic Saquinavir (**1**) (Ro 31-8959) as a development candidate in 1989 [1].

**Correspondence*: Dr. H. Hilpert
Pharmaceutical Division
Process Research
F. Hoffmann-La Roche Ltd.
CH–4070 Basel

CHIMIA 50 (1996) Nr. 11 (November)

Scheme 3



a) 1. H_2, Pd/C, THF; 2. + **31**, *N*-[3-(dimethylamino)propyl]-*N'*-ethyl-carbodiimide hydrochloride (EDC), DMAP, CH_2Cl_2. b) H_2, Pd/C, MeOH. c) *p*-TsCl, DMAP, CH_2Cl_2. d) $(Bu)_4NF$, THF. e) 1. H_2, Pd-C, THF; 2. HCl (g), i-PrOH, DME.

[7] R.J. Ferrier, N. Prasad, *J. Chem. Soc. (C)* **1969**, 570.
[8] R.J. Ferrier, *Methods Carbohydr. Chem.* **1972**, *6*, 307.
[9] M. Menard, L. Erichomovitch, M. La Brooy, F.L. Chubb, *Can. J. Chem.* **1963**, *41*, 1722.
[10] L.L. Chang, J. Hannah, W.T. Ashton, G.H. Rasmusson, T.J. Ikeler, G.F. Patel, V. Garsky, C. Uncapher, G. Yamanaka, W.L. McClements, R.L. Tolman, *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1207.

Chimia 50 (1996) 532–537

ISSN 0009–4293

Synthesis of the HIV-Proteinase Inhibitor Saquinavir: A Challenge for Process Research

Wolfgang Göhring, Surendra Gokhale, Hans Hilpert*, Felix Roessler, Markus Schlageter and Peter Vogt

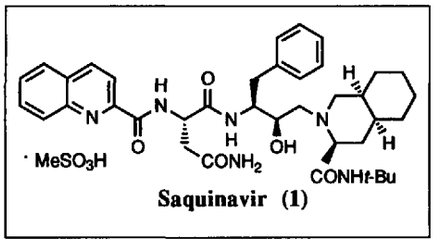
Abstract. The task of process research, namely developing efficient, economically and technically as well as ecologically feasible syntheses in time, is demonstrated on the HIV-proteinase inhibitor Saquinavir (**1**), a complex molecule comprising six stereocentres. Based on the first 26-step research synthesis furnishing a 10% overall yield, process research established a new, short 11-step synthesis affording a 50% overall yield.

1. Introduction

In 1986, the HIV-proteinase, an enzyme that catalyses the processing of the group antigen (gag) polyprotein p55 to the core proteins p24, p17 and p15, was recognized by *Kramer* [1] as a challenging new target to combat acquired immunodeficiency syndrome (AIDS). Subsequently, industry as well as academia started an intensive search for inhibitors of the HIV-proteinase. At our research laboratories in Welwyn, England, a number of potent inhibitors were synthesized and structurally optimized leading finally to the selection of the peptide mimetic Saquinavir (**1**) (Ro 31-8959) as a development candidate in 1989 [1].

**Correspondence*: Dr. H. Hilpert
Pharmaceutical Division
Process Research
F. Hoffmann-La Roche Ltd.
CH–4070 Basel

CHIMIA 50 (1996) Nr. 11 (November)



Saquinavir (1)

Saquinavir (**1**) was designed as a transition-state mimetic of the scissible Phe–Pro bond of the natural substrate – a cleavage site that is never found in substrates of human aspartic proteinases. Despite the high *in vitro* antiviral activity of Saquinavir (**1**) (IC_{50} *ca.* 2–10 nmolar) a dosage of 3 x 600 mg/day is required due to the rather low bioavailability of the compound. However, Saquinavir (**1**) was well tolerated in man, and the clinical program could be terminated uneventfully and according to plans. Subsequently, Saquinavir (**1**) was introduced as the first HIV-proteinase inhibitor onto the market under the trade name *Invirase*® in 1995. The benefit to patients too now has been demonstrated in a phase III clinical study in which *Invirase*® therapy resulted in a 72% decrease in incidence of death.

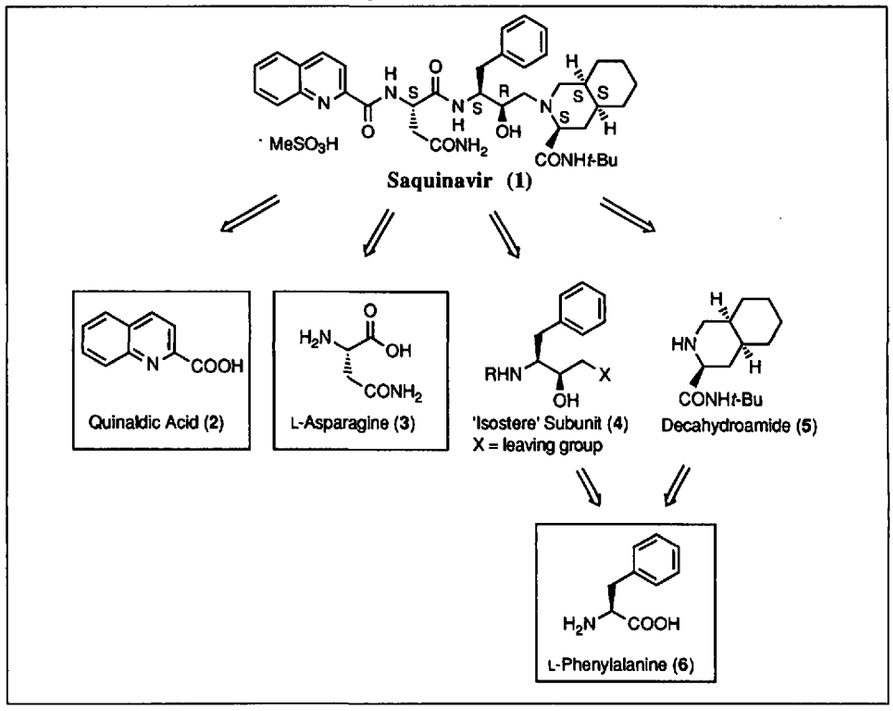
Successful drug development, on the one hand, relies on an innovative compound with good clinical efficacy and tolerability. On the other hand, an efficient synthesis is the prerequisite to support the clinical studies in a timely manner. Drug demand was a critical issue in this project primarily due to the structural complexity and the high substance demand requiring multiton quantities of Saquinavir (**1**). Process research was therefore challenged to developing a short, stereoselective and economic synthesis which should also be suitable for safe, large-scale production. The paper discusses the search for routes allowing a technically feasible production of Saquinavir (**1**).

2. The Research Synthesis

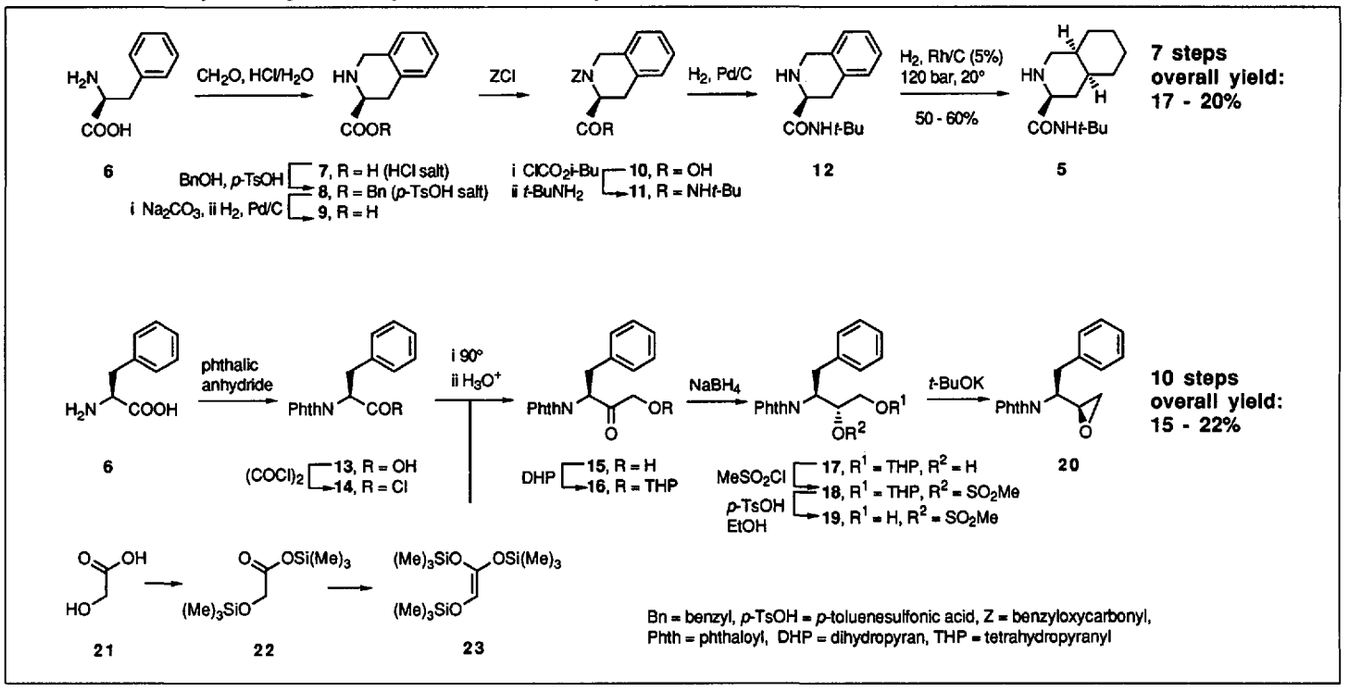
Retrosynthetically, the molecule can be dissected into the four building blocks (*Scheme 1*) quinaldic acid (**2**), L-asparagine (**3**), the 'isostere' subunit **4** and the decahydroamide **5**. Compounds **4** and **5** can be derived from L-phenylalanine (**6**).

The supply of the first kg amounts for the early development phase was achieved

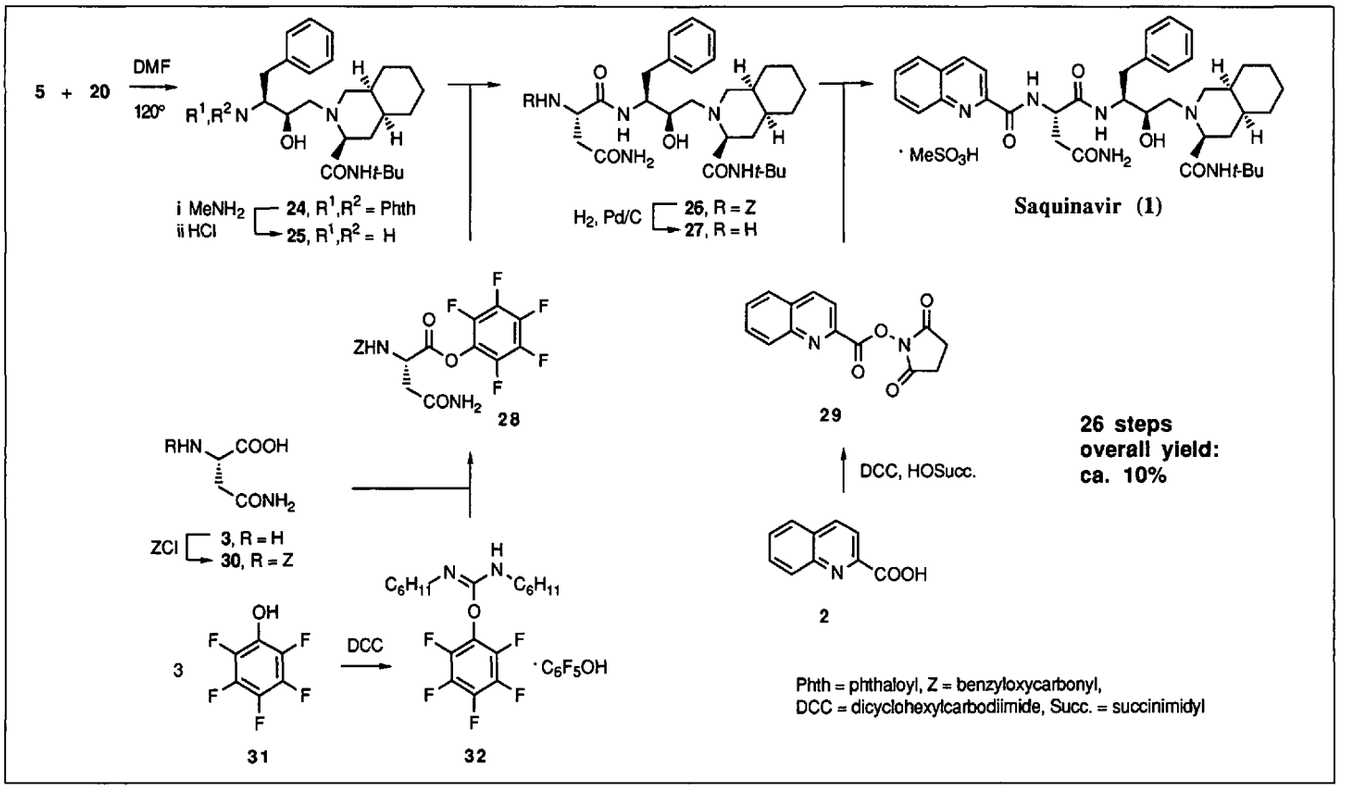
Scheme 1. *Retrosynthetic Analysis of Saquinavir* (**1**)



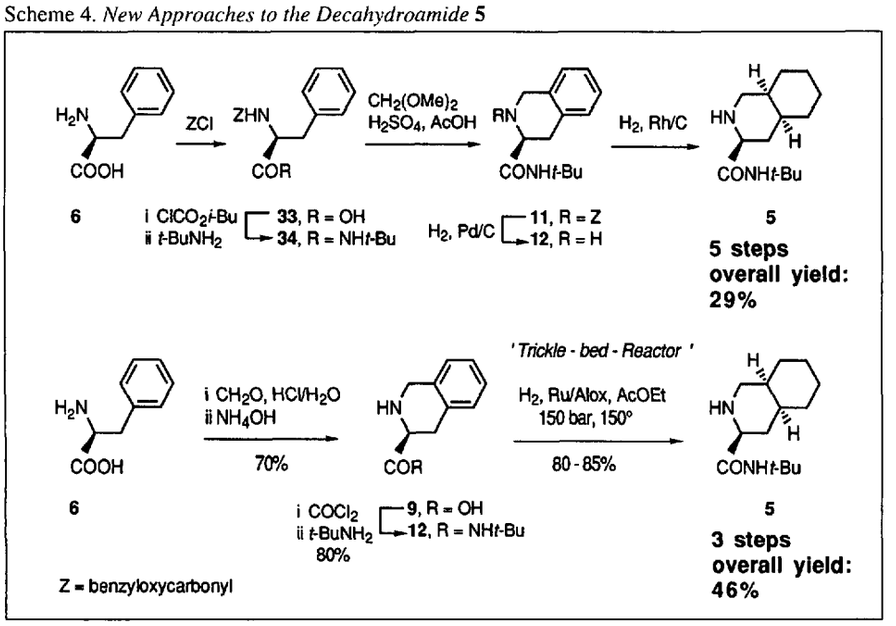
Scheme 2. *Research Synthesis of the Decahydroamide* **5** *and the Epoxide* **20**



Scheme 3. *Assembly of the Building Blocks* **5** *and* **20**



Scheme 4. *New Approaches to the Decahydroamide* **5**



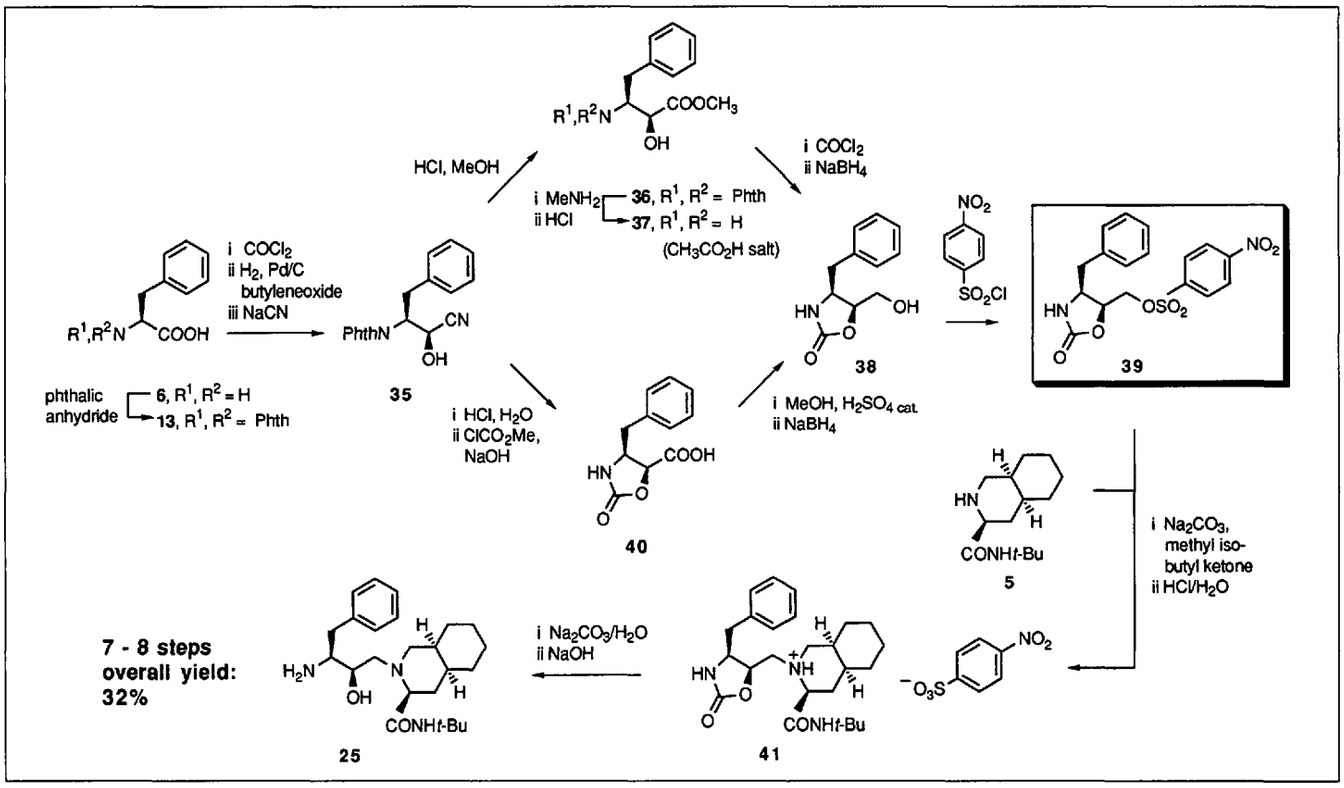
using a rather tedious 26-step process derived from the research synthesis [3] as outlined in *Schemes 2* and *3*.

Preparation of the decahydroamide **5** (*Scheme 2*) started with the *Pictet-Spengler* cyclization of L-phenylalanine (**6**) to tetrahydroisoquinoline-3-carboxylic acid (**7**). The harsh reaction conditions (100°) gave rise to partial racemization (*ca.* 15%) of the hydrochloride **7**, the required enantiomer of the mixture could not be separated. Reestablishing of the optical purity required purification *via* the crystalline *p*-TsOH salt of the benzylester **8** followed by hydrogenolysis to give enantiomerically pure **9**. Formation of the tetrahydroamide **12** was accomplished by hydrogenolysis of the amide **11** which in turn was prepared from the Z-protected acid **10** *via in situ* activation to the mixed anhydride. Finally, high pressure hydrogenation (*ca.* 100 bar) of the aromatic nucleus of **12** in the presence of a Rh catalyst produced a mixture of isomers out of which the desired (*S,S,S*)-configurated decahydroamide **5** could be isolated by crystallization. A total of seven steps led to **5** with an overall yield of 17–20%.

The phthaloyl-protected aminomethyl epoxide **20** (*Scheme 2*) served as the 'isostere' subunit **4**. The key step involves the C_1 homologization of the acid chloride **14** to the hydroxymethyl ketone **15** accomplished with the ketene acetal **23** developed by *Wissner* [4]. This conversion proceeded without a solvent and with some racemization (*ca.* 3%). Formation of the second chiral centre was accomplished *via* the THP-protected alcohol **16** and $NaBH_4$ reduction of the carbonyl group affording a 4:1 mixture of diastereoisomers containing primarily the desired *threo*-alcohol **17**. The sequence was completed *via* the mesylate **18**, cleavage of the THP-protecting group and cyclization of the hydroxy mesylate **19** to the epoxide **20** which was isolated in 98% optical purity and an overall yield of 15–22%.

Assembly of the building blocks **5** and **20** (*Scheme 3*) and deprotection of the phthaloyl group provided the amino alcohol **25**. The remaining 'left-half' part of **1** was introduced in a consecutive manner by amide bond formation of **25** with the pentafluorophenyl active ester **28** to give the Z-protected amide **26**. A final cou-

Scheme 5. *New Syntheses to the Nosylate* **39** *and Assembly to the Amino Alcohol 25*



pling of **27** with the active ester **29** furnished Saquinavir (**1**).

In terms of an economic large-scale production of Saquinavir (**1**) this 26-step synthesis was clearly unsatisfactory for various reasons: the two building blocks **5** and **20** are prepared by rather lengthy and low-yielding syntheses. The preparation of **5** was problematic as the *Pictet-Spengler* ring closure **6** → **7** caused partial racemization. Preparation of the epoxide **20**, essentially a C_1 homologization with control of one additional stereocentre, proceeds with minimal atom economy [5] by introducing ten superfluous C-atoms, involving the expensive ketene acetale **23** as evident by the requirement of 13 kg of **23** to produce 1 kg of the epoxide **20**. The overall yield of **20** was optimized (18% → 38%), but the number of steps was still very high. Finally, a more convergent process for the consecutive coupling of the asparagine and quinaldic-acid residue would be highly desirable.

3. New Approach to the Decahydroamide 5

To circumvent the racemization problem of the first step, a new approach to the decahydroamide **5** was developed whereby modified *Pictet-Spengler* conditions were elaborated and applied at a latter stage of the synthesis. Thus, the cyclization of Z-protected amide **34** (*Scheme 4*) to the tetrahydroamide **11** with formaldehyde dimethyl acetal and sulfuric acid in acetic acid proceeded under mild conditions (40°) and virtually free of racemization reducing the number of steps from seven to five and increasing the overall yield from 18% to 29% [6].

Further process research led to a short three-step sequence as outlined in *Scheme 4*. Ring closure of L-phenylalanine (**6**) could be effected with little racemization in the presence of a high concentration of HCl, which accelerated the rate of reaction markedly. Crystallization of the free amino acid **9** rather than the hydrochloride **7** provided optically pure material in 70% yield. The formation of the *N-tert*-butyl amide **12** proceeded *via* the *N*-carboxyanhydride intermediate thus providing simultaneously protection of the amine group and activation of the carboxy functionality. A remarkable further improvement on the hydrogenation of the aromatic nucleus of compound **12** was achieved with a Ru catalyst at high pressure (100–150 bar) and temperature (100–150°) providing up to 94% selectivity of the desired stereoisomer [7]. The forcing reaction conditions, not easily applicable in a batch reactor, were overcome by a continuous mode of reaction involving a '*trickle-bed-reactor*'.

4. Synthesis of the 'Isostere' Subunit 4 and Assembly to the Amino Alcohol 25

4.1. *Approach* via *Nosylate* 39

Efforts towards the preparation of a more economic, synthetically equivalent building block to the epoxide **20** led to a new synthesis targeting the nosylate **39** [8] as the key intermediate (*Scheme 5*).

Extension of the C-chain was achieved by a highly atom-economic method involving the formation of the cyanohydrin **35** prepared from the *N*-phthaloyl-protected phenylalanine **13** *via* the *in situ* generated acid chloride and aldehyde. The one-pot procedure furnished a 3:1 mixture containing preferentially the desired *erythro*-isomer **35** as predicted by a non-chelation control mechanism. Conversion of the *in situ* prepared acid chloride to the aldehyde by a *Rosenmund* reduction turned out to be nontrivial due to the sensitivity of the α-amino-acid chloride and aldehyde towards racemization. Neutralizing the hydrogen chloride (necessary to prevent inactivation of the catalyst) by amines was therefore unsuccessful, but butylene oxide as a hydrogen-chloride scavenger afforded the enantiomerically pure aldehyde [9]. *Pinner* reaction of the cyanohydrin **35** provided the hydroxy ester **36**, which could not be reduced selectively to the corresponding diol in the presence of the phthaloyl protecting group. Consequently, **36**

was deprotected and crystallized as the acetate salt **37** obtained in a 92% optical purity. Reprotection of **37** with phosgene and subsequent *in situ* reduction with $NaBH_4$ furnished the oxazolidinone **38**. Activation of the OH group of **38** with *p*-nitrophenylsulfonyl chloride provided the crystalline nosylate **39** in an overall yield of 40% from **6** and 99.5% optical purity.

A further shortening of the synthesis from six to five steps (41% overall yield [10]) was achieved *via* the oxazolidinone acid **40**, a compound that was readily obtained from **35** in 97% optical purity. This improved process substitutes phosgene by the safer methyl chloroformate, separates the desired *erythro*-isomer **40** more efficiently and at an earlier stage and uses water as solvent.

The nosylate **39** and the decahydroamide **5** were assembled in methyl isobutyl ketone at 80° affording the crystalline nosylate salt **41** in good yield (90%) and excellent quality by simple acidification of the reaction mixture. Hydrolysis of **41** led to the amino alcohol **25** in 7–8 steps [10] and an overall yield of 32%.

4.2. *Approach* via *Chlorohydrin* 44

The anticipated very large quantities of Saquinavir (**1**) required for the market led us to continue our search to further shorten the route to the amino alcohol **25**. A new route was designed involving a key intermediate with a lower molecular weight (less waste, more material convertible), which should allow to introduce the second chiral centre of the isostere subunit **4** with considerably higher selectivity compared to the approaches discussed in *Scheme 5*.

The methoxycarbonyl-protected ester **42** (*Scheme 6*), readily available from L-phenylalanine (**6**) in quantitative yield, was considered to be a suitable starting material.

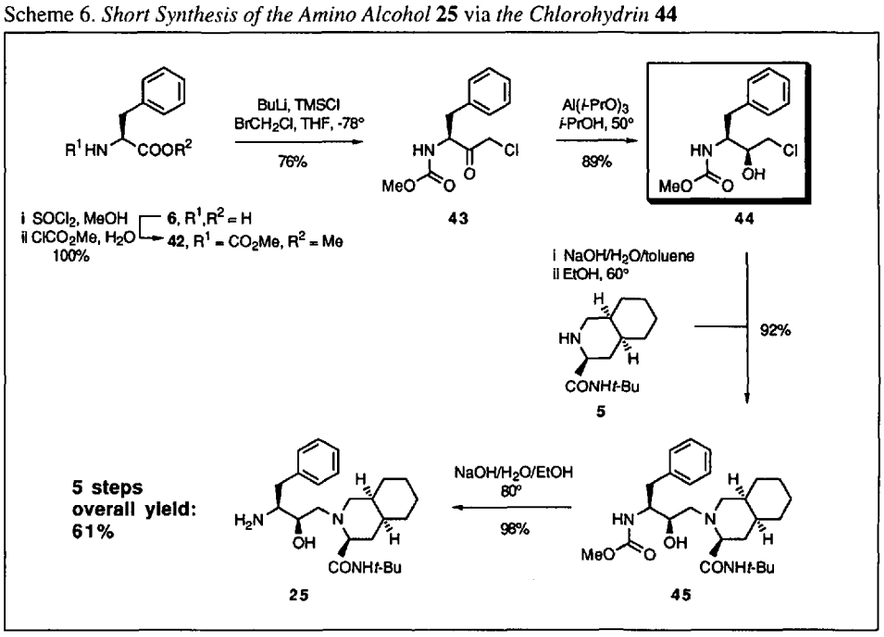
The key step entails the C_1 homologization of **42** to the chloromethyl ketone **43** by reaction of *in situ* generated chloromethyllithium at low temperature (–78°). Various conditions applied on other substrates [11] were tested, but afforded only modest yields (35–51%) of **43**. We assume that the *in situ* generated chloromethyllithium, well-known to be unstable even at low temperature, requires reactive esters. Since deprotonation of the carbamate group in **42** may lead to a cyclic hemiortho ester, such a deactivation of the ester group might account for the low yields. An improved process was elaborated by *in situ* protection of the carbamate group in **42** with trimethylsilyl chloride prior to the reaction with chloromethyllithium. During aqueous workup, hydrolysis of the TMS group occurred affording the chloromethyl ketone **43** in a significantly higher yield of 76%.

The reduction of the carbonyl group of **43** with $NaBH_4$ provided a modest selectivity of 3:1 in favour of the desired *erythro*-isomer **43**. An improved ratio of 95:5 was achieved with the method developed by *Corey* [12] involving the borane-THF complex in the presence of a chiral oxazaborolidine catalyst derived from α,α-diphenylprolinol and methylboronic acid or with $LiAlH(t\text{-}BuO)_3$ in EtOH (nonprotic solvents were not selective), but these reagents were considered to be too expensive for a large-scale manufacturing process. An excellent solution to the selectivity problem was found by the *Meerwein-Ponndorf-Verley* reduction (95% selectivity) involving cheap $Al(i\text{-}PrO)_3$ to give isomerically pure *erythro*-chlorohydrin **44** in 89% yield [13].

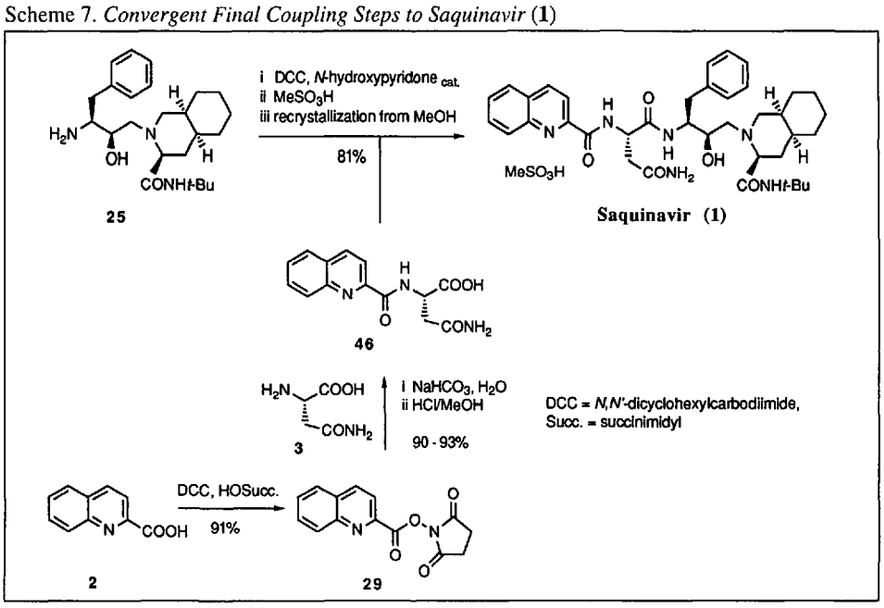
The new key intermediate **44** was cyclized *in situ* to the corresponding epoxide which was readily coupled with the decahydroamide **5** to the carbamate **45**. Hydrolysis of **45** afforded in almost quantitative yield the amino alcohol **25** [13].

The new chlorohydrin route approaches the amino alcohol **25** in only five steps and gives an almost double overall yield of 61% compared to the nosylate route (32%) meeting fully the aforementioned criteria set.

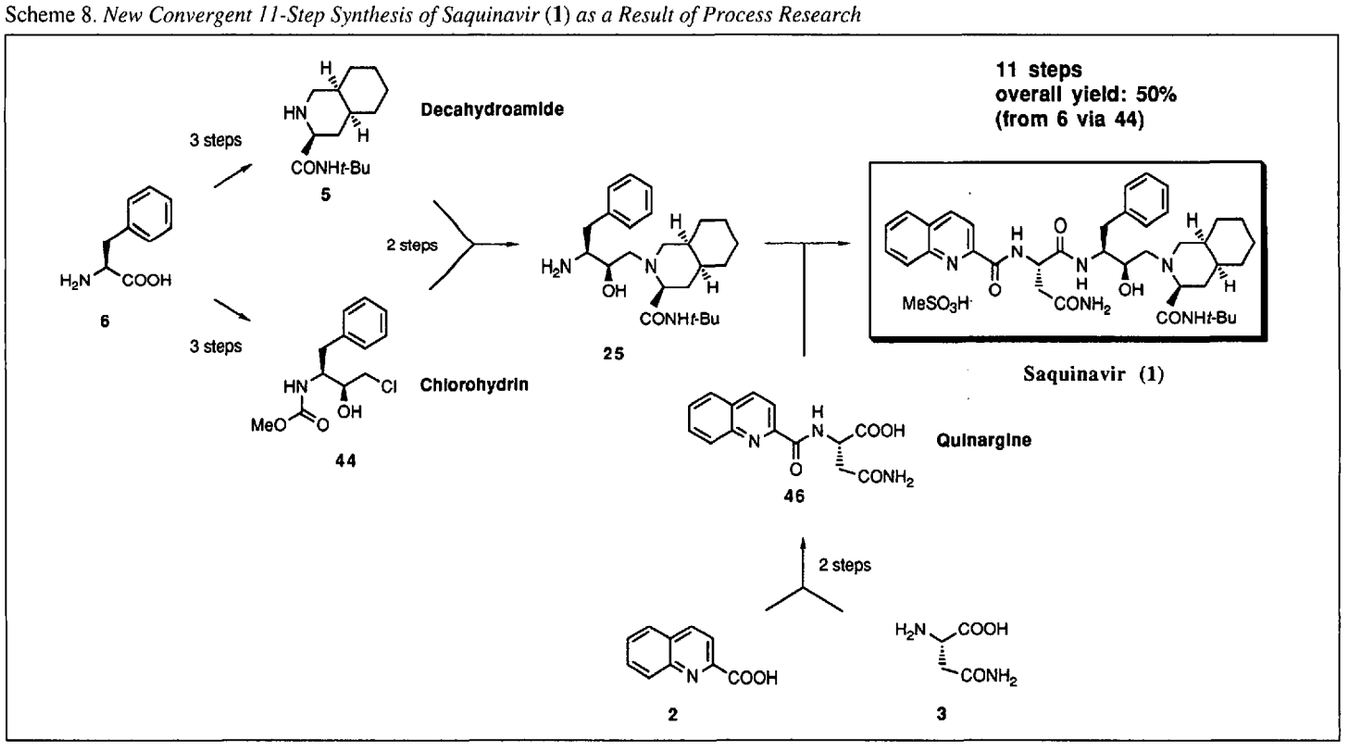
Scheme 6. *Short Synthesis of the Amino Alcohol* **25** via *the Chlorohydrin* **44**



Scheme 7. *Convergent Final Coupling Steps to Saquinavir* (**1**)



Scheme 8. *New Convergent 11-Step Synthesis of Saquinavir* (**1**) *as a Result of Process Research*



5. Convergent Final Coupling Steps

Having a short and high yielding route to the amino alcohol **25** at hand, we set out looking for a convergent coupling variant of **25** with the quinargine **46** (*Scheme 7*) to give Saquinavir (**1**). Compound **46** was efficiently prepared by reaction of the active ester **29** with L-asparagine (**3**) under neutral conditions in 90–93% yield [14].

The final coupling of the two segments **25** and **46** was not trivial due to the nitrile formation of the primary amide substituent in Saquinavir (**1**) and quinargine (**46**) by water-binding coupling reagents. After extensive development work, this coupling was efficiently achieved with *N,N'*-dicyclohexylcarbodiimide in the presence of 0.2 equiv. of *N*-hydroxypyridone [15]. Formation of the mesylate salt and recrystallization provided Saquinavir (**1**) in a very good yield of 81% and a high chemical and optical purity of 99.9%.

6. Conclusion

The challenge of developing a technical synthesis of the highly complex molecule Saquinavir (**1**) was tackled in a stepwise fashion. Thus, a first cycle of improvements of the synthetic route enabled us to provide rapidly large quantities of the drug required to initiate and maintain the clinical development program. As encouraging clinical results continued to emerge, it became apparent that for the success of this project an even more efficient route was crucial to prepare the very large quantities of **1** needed for the market. Starting with a 26-step synthesis (10% overall yield), continuous process research on a number of alternative routes resulted finally in a new, convergent and short 11-step synthesis (*Scheme 8*) affording a 50% overall yield (from **6** *via* **44**) of Saquinavir (**1**).

In conclusion, a good deal of the successful development of Saquinavir (**1**) is due to the crucial contributions of chemical process research and development described in this review. The results of this work enabled the plant to produce sufficient amounts of the compound to support, *e.g.*, the clinical studies without any delay. *Roche* could therefore make available Saquinavir (**1**) to AIDS patients in a very short period of time, *i.e.* about 6 years, and introduce *Invirase*® as the first HIV-proteinase inhibitor at the end of 1995.

We would like to thank all technicians, colleagues and collaborators who contributed to the successful development of Saquinavir (**1**). The reading of the manuscript by Drs. *Martin Karpf*, *Noel Roberts* and *Ulrich Widmer* is kindly acknowledged.

Received: September 10, 1996

[1] R.A. Kramer, M.D. Schaber, A.M. Skalka, K. Ganguly, F. Wong-Staal, E.P. Reddy, *Science* **1986**, *231*, 1580.

[2] N.A. Roberts, J.A. Martin, D. Kinchington, A.V. Broadhurst, J.C. Craig, J.B. Duncan, S.A. Galpin, B.K. Handa, J. Kay, A. Krohn, R.W. Lambert, J.H. Merrett, J.S. Mills, K.E.B. Parkes, S. Redshaw, A.J. Ritchie, D.L. Taylor, G.J. Thomas, P.J. Machin, *Science* **1990**, *248*, 358.

[3] K.E.B. Parkes, D.J. Bushnell, P.H. Crackett, S.J. Dunsdon, A.C. Freeman, M.P. Gunn, R.A. Hopkins, R.W. Lambert, J.A. Martin. J.H. Merrett, S. Redshaw, W.C. Spurden, G.J. Thomas, *J. Org. Chem.* **1994**, *59*, 3656.

[4] A. Wissner, *J. Org. Chem.* **1979**, *44*, 4617.

[5] B.M. Trost, *Angew. Chem.* **1995**, *105*, 285.

[6] M. Schlageter, S. Gokhale, Eur. Pat. Appl. EP 533,000, to *F. Hoffmann-La Roche AG*, 1992.

[7] F. Roessler, *Chimia* **1996**, *50*, 106.

[8] H. Hilpert, Eur. Pat. Appl. EP 635,493, to *F. Hoffmann-La Roche AG*, 1994.

[9] H. Hilpert, Eur. Pat. Appl. EP 559,034, to *F. Hoffmann-La Roche AG*, 1993.

[10] M. Kottenhahn, K. Drauz, H. Hilpert, Int. Pat. Appl. WO 96/07642 and WO 96/07634, to *Degussa AG* and *F. Hoffmann-La Roche AG*, 1995.

[11] J. Barluenga, B. Baragana, A. Alonso, J.M. Concellon, *J. Chem. Soc., Chem. Commun.* **1994**, 969; R. Tarhouni, B. Kirschleger, M. Rambaud, J. Villieras, *Tetrahedron. Lett.* **1984**, *25*, 835.

[12] E.J. Corey, S. Shibata, R.K. Bakshi, *J. Org. Chem.* **1988**, *53*, 2861.

[13] H. Hilpert, Eur. Pat. Appl. EP 710,651 and EP 703,209, to *F. Hoffmann-La Roche AG*, 1995.

[14] W. Göhring, Eur. Pat. Appl. No. 96,112,866, to *F. Hoffmann-La Roche AG*, 1996.

[15] P. Vogt, Eur. Pat. Appl. EP 611,774, to *F. Hoffmann-La Roche AG*, 1994.