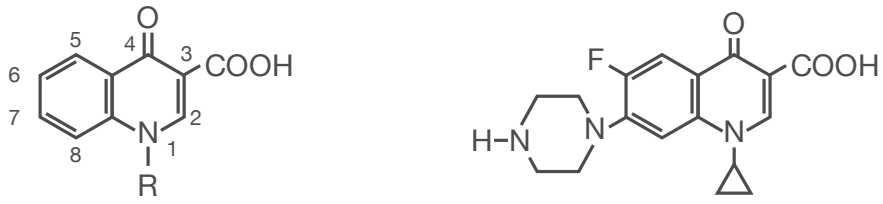
The Grohe method and quinolone antibiotics

Antibiotics are medicines that are used to treat bacterial infections. They contain active ingredients belonging to various substance classes, with modern fluoroquinolones one of the most important and an indispensable part of both human and veterinary medicine. It is largely thanks to Klaus Grohe – the "father of Bayer quinolones" – that this entirely synthetic class of antibiotics now plays such a vital role for medical practitioners. From 1965 to 1997, Grohe worked as a chemist, carrying out basic research at Bayer AG's main research laboratory (WHL) in Leverkusen. During this period, in 1975, he developed the Grohe process – a new multi-stage synthesis method for quinolones. It was this achievement that first enabled him to synthesize active antibacterial substances such as ciprofloxacin – the prototype for modern fluoroquinolones. The Grohe process and the synthesis of ciprofloxacin sparked Bayer AG's extensive research on fluoroquinolones and the global competition that produced additional potent antibiotics.

In chemical terms, the antibiotics referred to for simplicity as quinolones are derived from *1,4-dihydro-4-oxo-3-quinoline carboxylic acid (1)* substituted in position 1. Fluoroquinolones possess a fluorine atom in position 6. In addition, *ciprofloxacin (2)* has a cyclopropyl group in position 1 and also a piperazine group in position 7 **(Figure A)**. This substituent pattern plays a key role in its excellent antibacterial efficacy.



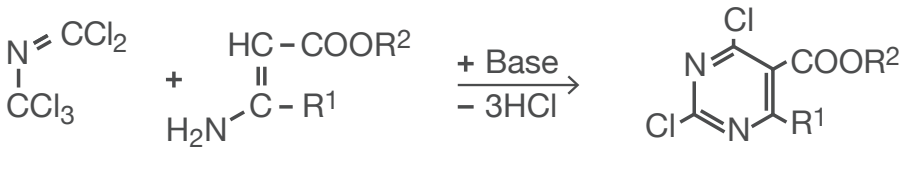
(1) (2)

Figure A: Basic structure of quinolone (1) (R = various substituents) and ciprofloxacin (2)

Quinolones owe their antibacterial efficacy to their inhibition of essential bacterial enzymes – DNA gyrase (topoisomerase II) and topoisomerase IV. The active substances prevent the bacterial DNA from supercoiling, meaning that the pathogens can no longer multiply and they ultimately die. In the medical context, quinolones are therefore also referred to as gyrase inhibitors.

This unique mode of action also makes fluoroquinolones highly effective against a large number of pathogenic bacteria that are resistant to penicillin, cephalosporin, aminoglycoside and tetracycline.

When Grohe started working as a chemist at Bayer AG's WHL in 1965, he initially focused on the synthesis of organochlorine and organofluorine intermediates. In 1969, at his own request, he transferred to the WHL's pharmaceutical research division. In the early 1970s, Grohe's basic research work led to the discovery of a general synthesis principle for 5- and 6-membered N-heterocycles known as cycloacylation. The cyclocondensation of polyfunctional acylating agents with tautomerizable enamines and enhydrazines enabled him to synthesize compounds such as pyrimidines, thiazolones, uracils and pyrazolium betaines. Cycloacylation of *trichloromethyl isocyanide dichloride (3)* with *enamino esters (4)*, for example, produced *pyrimidine carbon esters (5)* **(Figure B)**.

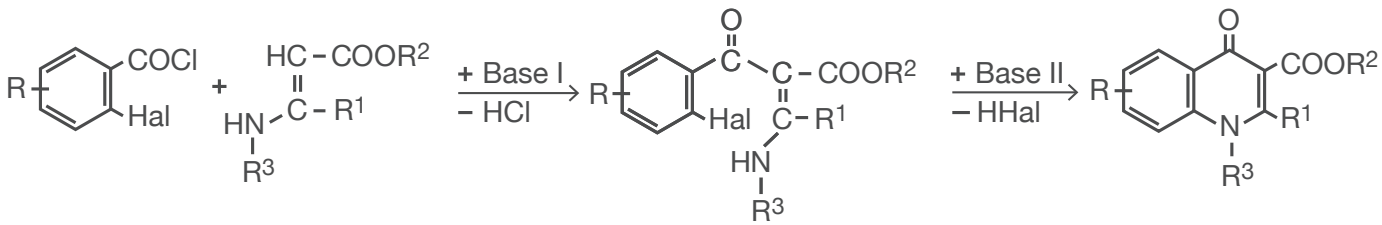


(3) (4) (5)

Figure B: Synthesis of pyrimidine carbon esters (5) using cycloacylation

Despite all kinds of further pharmaceutical and crop protection research on numerous derivatives of these heterocycles, there was no breakthrough that resulted in a marketable product.

In 1975, Grohe modified the general reaction principle, using *o-halogenated benzoyl chlorides (6)* as a cyclocondensation agent for the first time. Based on combined acylation and arylation with *secondary enamino esters (7)* by way of *intermediates (8)*, he thus obtained the *quinolone carboxylic acid esters (9)* in a completely new way.



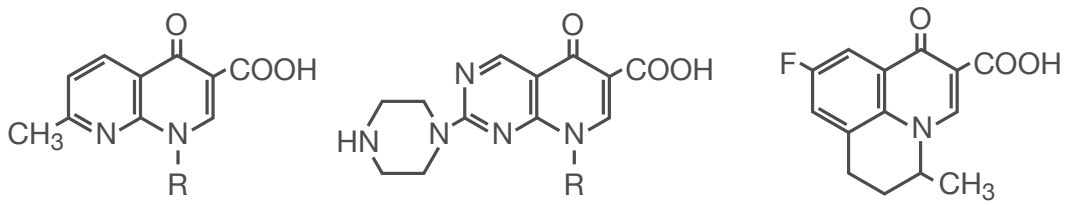
(6) (7) (8) (9)

Figure C: Grohe process (Hal = Cl, Br)

Known as cycloaracylation **(Figure C)**, this approach was subsequently referred to in literature as the Grohe process or Grohe method.

Grohe was keen to continue his work on quinolones, so that same year the new head of research transferred him from the WHL's pharmaceutical research division to the crop protection team, where he was able to pursue this research alongside his other activities.

As someone who was passionate about experimenting, Grohe was spurred on by the many different new chemical possibilities of his method when compared with the traditional Gould-Jacobs reaction. Associated documentary research carried out by Grohe, during which he came across *nalidixic acid (10)*, *pipemidic acid (11)* and *flumequine (12)* **(Figure D)**, also led him to believe that the quinolones he had produced offered huge medical and pharmaceutical potential.



(10) (11) (12)

Figure D: Nalidixic acid (10, R = ethyl), pipemidic acid (11, R = ethyl) and flumequine (12)

The antibacterially effective *azaquinolone carboxylic acids (10, 11)* and tricyclic *quinolone carboxylic acid (12)* all have a similar structure to the compounds Grohe synthesized. Since they have only a relatively weak antibiotic effect against gram-negative bacteria, exhibit poor oral absorption and lead to a rapid development of resistance, however, they were primarily used to treat urinary tract infections.

Grohe wanted to use his new process, which synthesized quinolones with a completely new kind of substituent pattern, to improve these properties and obtain a quinolone antibiotic that was suitable for systemic use. The experienced chemist was particularly interested in new chemical groups in position 1, that is to say on the nitrogen atom. Having an ethyl group in this position, as in *nalidixic acid (10)* or *pipemidic acid (11)*, had been regarded as optimum for the antibacterial effect up to that point.

Due to their size and also for sterical and energy reasons, however, Grohe felt a cyclopropyl group was a more promising alternative. It also occurred to him that he had not come across any mention of 1-cyclopropyl quinolone carboxylic acids in his documentary research. He soon discovered why – it was impossible to produce compounds of this kind using methods such as the Gould-Jacobs reaction.

It was, however, perfectly possible to do so with the Grohe method, as he was able to demonstrate using cyclopropyl variants of *nalidixic acid (10)* and *pipemidic acid (11)* (R = cyclopropyl) as an example. As he had hoped, the antibacterial efficacy of both variants was far superior to that of the comparable compounds. Unfortunately, this clear progress was not sufficient to justify further development at the time.

Discovering that a cyclopropyl group in position 1 can significantly improve antibacterial efficacy did, however, convince Grohe he was on the right track – and that encouraged him to continue his research work.

His breakthrough came in the late 1970s when – initially on paper – he combined the basic quinolone structure with the cyclopropyl group in position 1, the fluorine atom in position 6 and the piperazine group in position 7. On November 20, 1979 – long before the arrival of norfloxacin from Japanese company Kyorin – he thus became the “father of cyclopropyl fluoroquinolones” by devising the structural formula of ciprofloxacin.

Neither a serious illness nor a decision by the company's Pharma Division in September 1980 to halt work on quinolones prevented him from using the Grohe process to synthesize ciprofloxacin (2, R = H) on April 15, 1981 and enrofloxacin (2, R = ethyl) three days later **(Figure E)**.

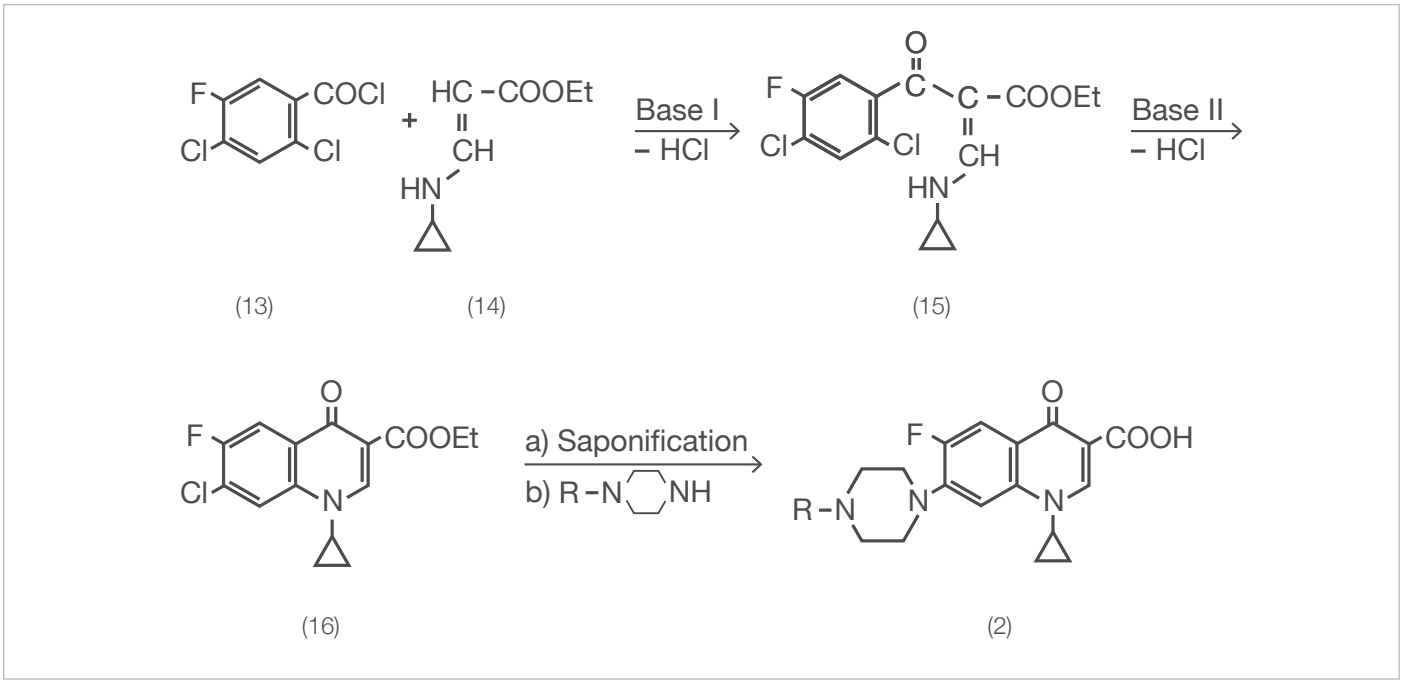


Figure E: Synthesis of ciprofloxacin (2, R = H) and enrofloxacin (2, R = ethyl) using the Grohe (cycloaracylation) process

Since the *enamino ester (14)* was not easy to produce and only provided moderate yields of the *intermediate (15)* with *(13)*, Grohe invented two further reaction sequences for the production of *(15)* **(Figure F)**. This significantly broadened the scope for varying his method on both a laboratory and an engineering scale.

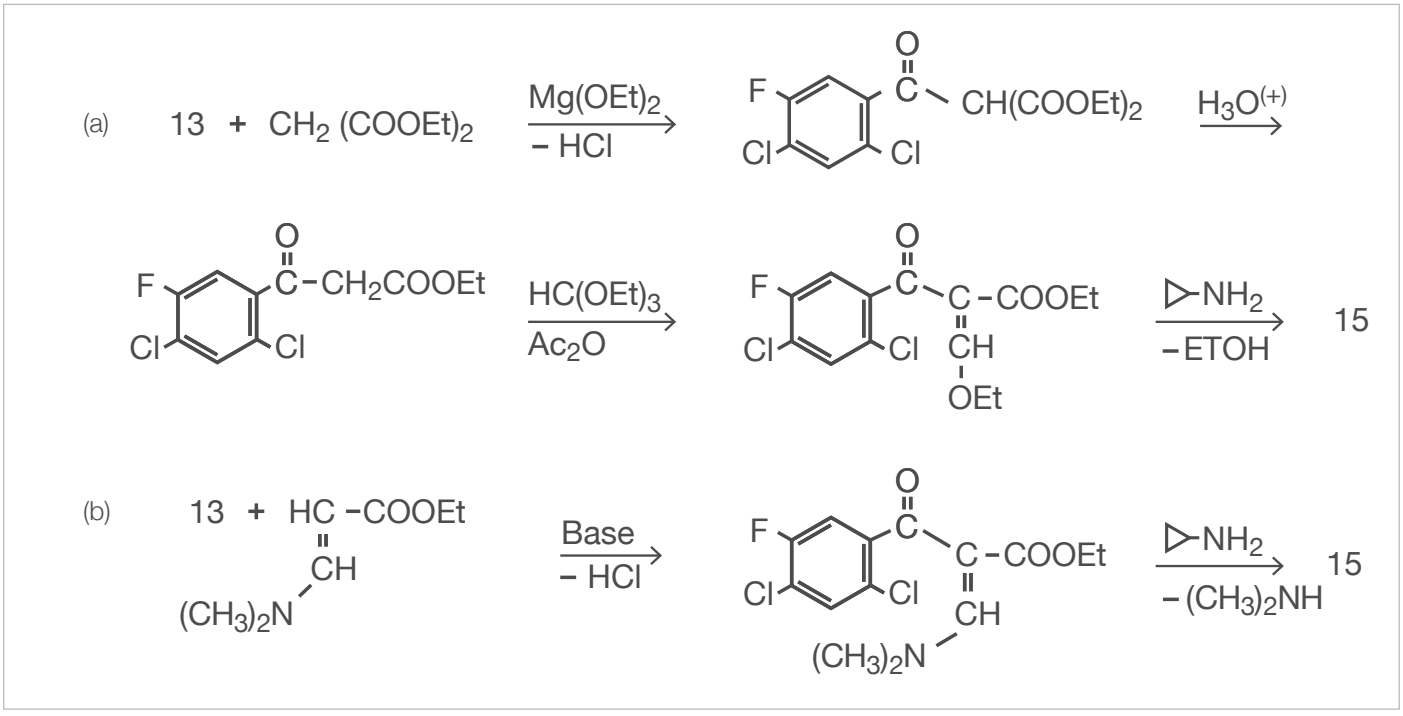


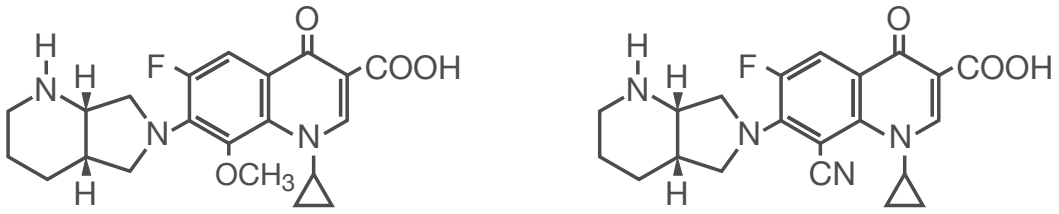
Figure F: Malonic ester (a) and dimethylamino acrylester (b) variants of the Grohe process for producing the central intermediate (15)

Ciprofloxacin is an exceptionally broad-spectrum antibiotic. In addition to gram-negative bacteria – including the Pseudomonas aeruginosa pathogen, which is difficult to treat – it is also effective against gram-positive bacteria. Excellent pharmacokinetics, rapid absorption from the gastrointestinal tract and outstanding tissue penetration mean ciprofloxacin can be used to treat a whole host of systemic infections. It was also the first quinolone antibiotic that could be administered both orally and parenterally.

In 1987, Bayer launched ciprofloxacin as a broad-spectrum antibiotic in Germany, the United Kingdom and the United States. Enrofloxacin followed in 1988 for veterinary use. By 1992, ciprofloxacin's exceptional antibacterial properties had already made it the world's best-selling antibiotic and the gold standard in fluoroquinolones. It has now been used to successfully treat many hundreds of millions of people. The antibiotic garnered international attention in 2001 when anthrax pathogens were used as a biological weapon in the United States. Ciprofloxacin was the only drug approved to combat this dangerous disease at the time, and it was successfully used for both prevention and treatment.

The fight against pathogenic bacteria never stops, though. Following the synthesis of ciprofloxacin in 1981, the next step was to make quinolones even more effective, especially in the gram-positive range. With Grohe acting as project manager, providing guidance and assisting with experiments, a slowly growing team set about systematically modifying the ciprofloxacin molecule's substituents. Functionalizing position 7 with cyclic diamines was a particular focal point.

In 1988, following several development products that raised high hopes but failed as a result of toxicological problems, the team produced *moxifloxacin (17)*, which met the above-mentioned objectives and was launched by Bayer in 1999 to treat bacterial respiratory diseases **(Figure G)**. Moxifloxacin is particularly effective against gram-positive bacteria such as streptococci and pneumococci, but also against atypical pathogens including chlamydia, mycoplasma and legionella. In addition to successfully treating community-acquired pneumonia, bronchitis and sinusitis, this antibiotic is also prescribed for tuberculosis patients.



(17) (18)

Figure G: Moxifloxacin (17) and pradofloxacin (18)

In the early 1990s, the work on quinolones resulted in the discovery of another active substance – *pradofloxacin (18)* – which is mainly used to treat cats and dogs with bacterial infectious diseases **(Figure G)**.

Back in the 1970s and 1980s, Grohe brought about the evolution of fluoroquinolones, especially cyclopropyl fluoroquinolones, with the Grohe process and the synthesis of both ciprofloxacin and enrofloxacin at Bayer AG's WHL in Leverkusen.

The variants of his innovative process could be used to produce not only the well-known nalidixic acid (1st generation) quinolones, but also, for the first time, the modern ciprofloxacin (2nd generation) and moxifloxacin (3rd generation) cyclopropyl fluoroquinolones, which cannot be obtained using the traditional Gould-Jacobs reaction.

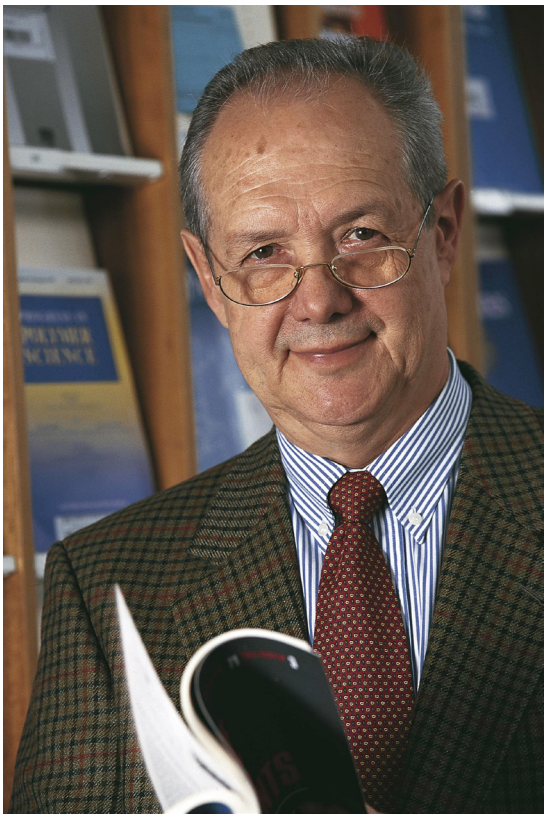
By making it possible to introduce many other substituents besides the cyclopropyl group into the effect-sensitive position 1 of quinolone carboxylic acids (phenyl, 4-fluorophenyl, 2,4-difluorophenyl, hetaryl, tert-butyl, etc.), the Grohe process provided huge impetus for quinolone research around the world. This was reflected in a flood of publications, patents and presentations at international congresses.

Global competition gave rise to a large number of fluoroquinolone development products based on the Grohe process. Examples include gatifloxacin, gemifloxacin, finafloxacin, besifloxacin and delafloxacin in human medicine, and also difloxacin, danofloxacin and orbifloxacin in veterinary medicine. Just like ciprofloxacin, moxifloxacin, enrofloxacin and pradofloxacin, some of these active ingredients have become indispensable to doctors, vets and hospitals.

Furthermore, there are many clear signs in relevant literature that quinolone carboxylic acids and quinolone carboxylic acid amides could play a key role in future Helicobacter pylori, antimycobacterial, antiviral, antitumor and antiplasmodial research. The Grohe method is definitely the "tool" of choice for this upcoming research work.

About Klaus Grohe:

Born in the German city of Ludwigshafen in 1934, Professor Klaus Grohe studied chemistry and medical chemistry at the University of Würzburg. Having obtained a doctorate in organic chemistry in 1964, he spent nearly two years as a research assistant before starting work at Bayer AG's main research laboratory (WHL) in Leverkusen at the end of 1965. In 1975, he invented the Grohe method, which enabled him to synthesize the active antibacterial substances ciprofloxacin and enrofloxacin for the first time. This was the catalyst for the quinolone research at Bayer that went on to produce the quinolone-based active substance moxifloxacin and, subsequently, pradofloxacin, also under his guidance.



In 1987, Grohe was presented with the Otto Bayer Medal in recognition of his work on ciprofloxacin. Even though he retired in 1997, the "father of Bayer quinolones" was awarded the Otto Bayer Medal again in 2001, this time in honor of his life's work. No one else has as yet received this accolade twice. In 2005, Grohe was presented with the Order of Merit of the Federal Republic of Germany, 1st Class. In 2010, the State Government of North Rhine-Westphalia conferred upon him the title of professor in recognition of his outstanding scientific achievements.

Shortly after the start of the new millennium, Grohe and his wife set up two foundations to support young scientists working in the fields of medical active substance research and infectiology.