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# Aaptamine and related products. Their isolation, chemical syntheses, and biological activity

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## 1. Introduction

The marine environment is home to an incredible biodiversity in both flora and fauna. Soft-bodied sessile marine invertebrates constitute the largest biomass of the marine macrofauna in many marine habitats. During evolution, the diverse marine ecosystems offered different stimuli and challenges to organisms that lived in it; in order to survive in these highly competitive environments, over millions of years the invertebrate community has evolved complex bioactive chemistries, primarily for communication, reproductive and defensive purposes.[1]

Therefore, the storage of biologically active secondary metabolites by marine invertebrates is frequently related to their ecological success, despite of their exposition to fouling, predation, infestation by microbial pathogens, overgrowth, and competition for space and nutrients.[2] Moreover, these organisms learned to live in complex symbiotic associations, where transfer of nutrients and other chemicals between the partners, often difficults the discovery of the real origin of the produced metabolites.

Marine natural products, compounds produced by marine plants, invertebrates, and microorganisms are of unprecedented chemical diversity.[3] In addition, many of them have new and unusual structures that have not been encountered in studies of terrestrial organisms, and also often exhibit significant biological activities.[4] The tremendous diversity of structurally unique compounds, produced by marine invertebrates, particularly those characterized for their toxicity,[5] has strongly stimulated marine natural product research.[6]

During the past 25 years, hundreds of novel compounds have been isolated from various marine sources.[7–9] The so-called marine pharmacy currently holds more than 35,000 biological samples, with at least a dozen candidates being currently in various phases of human clinical trials, only for treatment of different cancers.[10] Interstingly, it is currently considered that sponges are among the best sources of novel compounds, also displaying the greatest occurrence of potential pharmaceuticals.[11]

The chemical defenses of sessile marine invertebrates may not only possess a specific ecological or physiological role, but may also exert a multitude of biological activities. Sponges and ascidians produce nitrogen-containing substances, usually called marine alkaloids, the study of which has profoundly influenced the course of discovery in the field of pharmacology, specially oncology.[12,13] These compounds have been found to interact with key aspects to the cell cycle, and with enzymes or other targets, providing insights into new therapeutics, including antibacterial, anticoagulant, antiviral, antifungal, antiinflammatory, antituberculosis, antimalarial, and antiplatelet agents,[14] and the hope of new cures to important existing diseases.

Aaptamine (**1**) and its natural congeners, collectivelly known as 'aaptamines', are marine alkaloids, which contain a benzo[*de*][1,6]-naphthyridine framework. All the known aaptamines have been isolated from Demospongiae (Table 1), one of the four classes of the Porifera phylum. Porifera are considered the oldest and structurally simplest metazoan phylum, while Demospongiae (divided in three sub-classes) comprises the largest, commonest, and most widely distributed class of sponge species.

The genus *Aaptos* (Hadromerida, Suberitidae) is cosmopolitan and currently about 20 species have been described. Although producers of different bioactive substances,[15] they are mostly known for their ability to be a rich, although not exclusive, source of 1*H*-benzo[*de*][1,6]-naphthyridine alkaloids (aaptamines). On the other hand, sponges of the genus *Xestospongia* (Haplosclerida, Petrosiidae) have proven to be a rich source of alkaloids, polycyclic quinones, and hydroquinones, polyacetylenic derivatives, aminoalcools, heterocyclic compounds, and original sterols.[16] Some of these compounds display significant cytotoxic,[17] antimicrobial or vasodilatory activity. The genus *Suberites* (Hadromerida, Suberitidae), related to *Aaptos*, has been shown to produce structurally complex terpenoids and bioactive peptides.[18] The genus *Luffariella* (Dictyoceratida, Thorectidae) is also the source of several interesting bioactive compounds.[19] In addition, the genus *Hymeniacidon* (Halichondrida, Halichondriidae)[20] produce brominated

**Table 1**
Isolation of aaptamine and congeners. Sponge sources and places, in an approximate chronological order

| Source (sponge) | Place and date of collection or disclosure of the isolation | Isolated compounds | Ref. no |
|---|---|---|---|
| *A. aaptos* | Manza beach, Okinawa (1981) | **1**, **3**, **6** | 26,27 |
| *Suberites* sp. | Indian Ocean (1985) | **1**–**3** | 28 |
| *Suberites* sp. | Darwin Harbour, Northern Australia | **1** | 29 |
| *A. aaptos* | Red sea (1990) | **1**–**3**, **6**, **18** | 30 |
| *Luffariella* sp. | Manado Bay, Sulawesi, Indonesia (1992) | **1**, **6** | 31 |
| *Hymeniacidon* sp. | Terumbu Pemalang Besar Reef, Singapore (1989 and 1992) | **1**, **2**, **6**, **11** | 42 |
| *Aaptos* sp. | Abrolhos, Bahia, Brazil (1995) | **6**, **10** | 37 |
| *A. aaptos* | Macqueripe Bay (1984) and Rust Bay (1996), Trinidad, Southern Caribbean | **3**, **19** | 35 |
| *Suberea* sp. | Lihou Reef, Coral Sea, New Caledonia (1996) | **16** | 51 |
| *A. suberitoides* | Indonesia (1996) | **1**, **2**, **6**, **7** | 34 |
| *A. aaptos* | Taiwan (1997) | **1**, **2**, **6**, **18** | 33 |
| *Aaptos* sp. | Manado, Northern Sulawesi, Indonesia | **1** | 36 |
| *A. suberitoides* | Spermonde Archipelago, Southern Sulawesi, Indonesia | **1**, **3** | 23a |
| *Xestospongia* sp. | Jakarta, Indonesia (2001) | **1**, **2**, **6**, **11**–**15** | 12,38 |
| *Aaptos* sp. | Vietnam | **1**-**3** | 39 |
| *A. suberitoides* | Carita, West Java Island, Indonesia (2003) | **1** | 45 |
| *Aaptos* sp. | Bunaken Island, Northern Sulawesi, Indonesia (2004) | **1**–**5** | 43 |
| *A. nigra* Lévi, 1961 | Manado Bay and Derawan Island, Indonesia (2005) | **1**, **2** | 46,47 |
| *A suberitoides* | Bali Island, Indonesia (2005) | **1**, **3** | 44 |
| *A. aaptos* | Chuuk Atoll, Federated States of Micronesia (2005) | **1**–**3**, **6** | 48 |
| *A. aaptos* | Coast of Terengganu, Eastern Peninsular Malaysia (2009) | **1**, **8**, **9** | 49 |

heterocycles and alkaloids, while marine sponges belonging to the genera *Suberea* and *Aplysina* (Verongida, Aplysinidae) are considered the major sources of bromotyrosine derived alkaloids, compounds, that often exhibit potent biological activities.[21]

Finding the bioactive compounds in sponges is relatively easy, and possibilities for prospecting still appear immense; however, the drug development process requires such large amounts of metabolites that may cause extinction of the studied sponge species.[11,22] In order to guarantee sustainability of the natural sources, different solutions have been proposed, including sponge farming, primmorph systems, and biosynthesis.[23] Optimization of these approaches is another incentive to sponge research.

## 2. The isolation of aaptamine and congeners

Curiously, the benzo[*de*][1,6]naphthyridine framework characteristic of the aaptamines was studied theoretically by the group of Efros[24] a few years before being first found in nature. Natural products having dimeric, rearranged, and differently functionalized benzo[*de*][1,6]naphthyridine skeletons, including those bearing a fourth ring, have all been isolated from marine sponges. Interestingly, however, the dibenzonaphthyridinone alkaloid necatorone (**20a**), a highly mutagenic pigment isolated from the fruit-bodies of the gilled toadstool *Lactarius necator* (Agaricales), its dimers **20b**,**c** isolated from the same source,[25a–e] and **20d** the main responsible for the green appearance of the North American species *Lactarius atroviridis*, which also contains **20a**–**c**,[25f] also exhibit the same basic skeleton.

The isolation of aaptamine (**1**), the first and most representative member of this family (Fig. 1) was initially reported by Nakamura et al.[26] from the marine sponge *Aaptos aaptos* Schmidt 1864, collected off the shores of Okinawa. Five years later, this group disclosed the isolation of two additional aaptamines 9-demethylaaptamine (**3**) and demethyl(oxy)-aaptamine (**6**)[27] from the same sponge. An approximately chronological detail of the isolation of aaptamine and its different congeners is shown in Table 1.

The group of Fedoreev[28] unveiled in 1988 the isolation of **1**, together with **3** and isoaaptamine (**2**) from a *Suberites* sp. sponge (Hadromerida, Suberitidae, sub-class Tetractinomorpha), collected in the Indian Ocean in 1985. Compound **1** was reported again by Bergquist et al. from an Australian *Suberites* sp. sponge,[29] in 1991. Compounds **1**–**3** and **6** were again isolated in 1993 by Kashman et al.[30] from *A. aaptos* collected in the Red Sea, together with the rearranged 8*H*-5,8-diazabenz[*cd*]azulene alkaloid aaptosine (**18**), while in 1995 the group of Park isolated the widespread compound **1** from a *Luffariella* sp. sponge collected in Manado Bay (Sulawesi, Indonesia);[31] the next year, these Korean scientists also disclosed the isolation of **6** from the same sponge.[32]

In 1997, compounds **1**, **2**, **6**, and the rearranged tricycle **18** were isolated by Shen et al. from *A. aaptos* collected in Taiwan;[33] the same set of benzo[*de*][1,6]naphthyridines were also obtained from *Aaptos suberitoides* specimens, collected in 1996 in Indonesia, together with **7**. Presumably, the latter is an artifact produced during the initial methanolic treatment of the sponge.[34] On the other hand, Tinto reported the isolation of aaptosamine (**19**) from *A. aaptos*, collected in the Southern Caribbean, probably an artifact resulting from capture of acetone (the extracting solvent) or an equivalent related compound, by **18**; the isolation of **1** from an earlier collection of the same sponge was also informed.[35]

Employing LC–NMR techniques, in 2000 Bobzin et al. identified **1** as the active component in the crude dichloromethanic extract of *Aaptos* sp. collected in Manado (Indonesia),[36] while the presence of **1** and **3** in *A. suberitoides* collected in the Spermonde Archipelago (South Sulawesi, Indonesia) was also informed.[23] As a result of studying *Aaptos* sp. specimens from Abrolhos (Bahia, Brazil) in

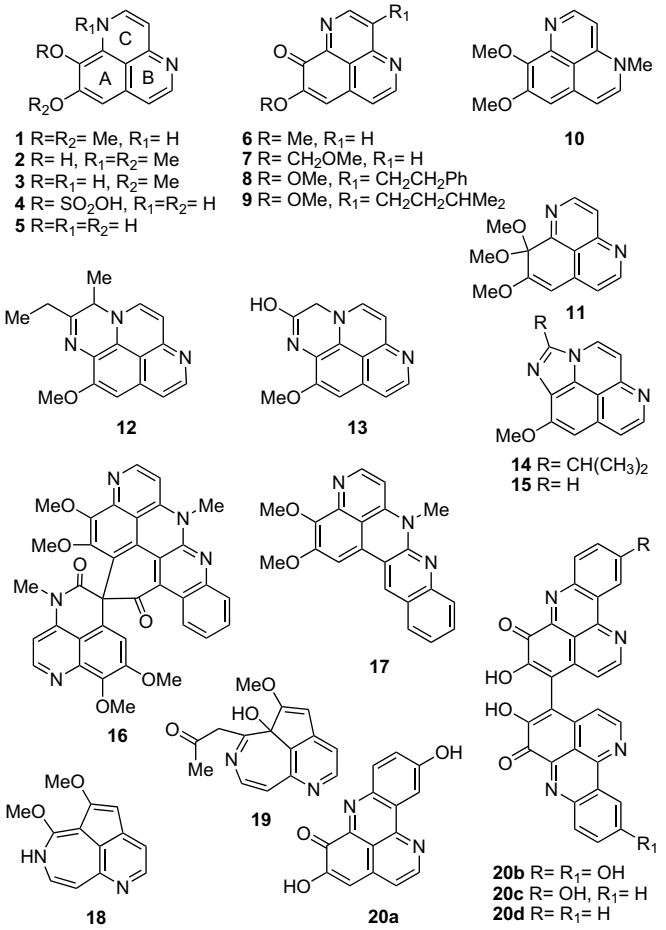



**Figure 1.** Chemical structures of aaptamine (**1**), its tricyclic (**2**–**11**) and tetracyclic (**12**–**15**) congeners; the bis-aaptamine derivative lihouidine (**16**) and its pentacyclic core (**17**), the rearranged aaptamines aaptosine (**18**) and aaptosamine (**19**), and necatorone (**20a**) and its dimers (**20b**–**d**).

1995, Coutinho et al.[37] found 4-methylaaptamine (**10**), together with known compound **6**.

In 2003, Calcul et al.[38] isolated ketal **11** and four new tetracyclic alkaloids (**12**–**15**) related to the aaptamines, in addition to the known tricycles **1**, **2**, and **6**, from the Indonesian marine sponge *Xestospongia* sp. collected off Jakarta. The next year, compounds **1**–**3** were isolated from *Aaptos* sp. collected in Vietnam.[39]

Bergquist et al. proposed aaptamine as a taxonomic marker for the Suberitidae sponges, within the order Hadromerida (sub-class Tetractinomorpha).[29] However, isolation of aaptamine in large quantity from *Xestospongia* sp.,[38] a taxonomically unrelated sponge of the order Haplosclerida (sub-class Ceractinomorpha), together with a case of failure to obtain 1*H*-benzo-[*de*][1,6]-naphthyridine alkaloids from an *Aaptos* sp. specimen collected at the Prainha Island (Saint Sebastian Channel, Brazil),[40] led to the conclusion that aaptamine and related compounds are not faithful chemotaxonomic markers for these sponges.[41]

The group of Pettit,[42] which would became heavily involved in studying the chemistry and biological properties of aaptamines, disclosed in 2004 their isolation of **1** and **6** from a *Hymeniacidon* sp. sponge collected at Terumbu Pemalang Besar Reef (Republic of Singapore) in 1989 and 1992. This was accompanied by isolation of traces of **2** and methoxymethyl ether **7**, previously isolated by the group of Calcul and likely to be an artifact formed during the isolation process. On the other hand, Herlt et al.[43] disclosed in 2004 the isolation of two new aaptaminoids, bisdemethylaaptamine (**5**) and bisdemethylaaptamine-9-*O*-sulfate (**4**), besides the known

compounds **1**–**3**, from *Aaptos sp.* harvested near Bunaken Island (North Sulawesi, Indonesia).

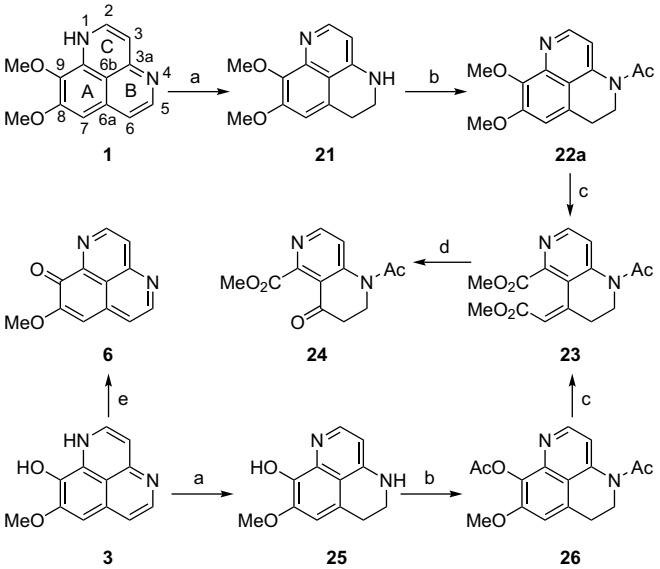
Later on, in 2005, Pedpradab unveiled the obtention of **1** and **3** from *A. suberitoides* specimens from Bali Island (Indonesia)[44] and Aoki et al.[45] isolated **1** from *A. suberitoides* collected at Carita (West end of Java Island, Indonesia), while Diers et al.[46,47] obtained compounds **1** and **2** from *Aaptos nigra* Lévi 1961, collected at Manado and Derawan Island (Indonesia). Finally, in 2007, Jang et al.[48] isolated aaptamines **1**–**3**, together with **6** as a minor constituent, from *A. aaptos* collected at the Chuuk Atoll (Federated States of Micronesia).

Very recently, Shaari et al. unveiled the structures of two new aaptaminoids, the first ones carrying substituents at C-3, which were isolated from a Malaysian *A. aaptos* employing a bioassay guided fractionation of the methanolic extract of the sponge.[49]

Since aaptamine and related compounds were isolated from different genera (*Aaptos*, *Suberites*, *Luffariella*, *Xestospongia*, and *Hymeniacidon*), which are usually found in different geographic locations and because of the distant relationship between these sponges, it was hypothesized that the source of the aaptamines within these sponges may be a symbiotic microbe that is common between their genera. Efforts have been made to characterize these microbial communities;[50] however, to date, these seem to be still unsuccessful. Interestingly, quinolones have been reported from a bacterium cultured from *Suberea creba* collected in New Caledonia,[21d] reinforcing the theory about the bacterial origin of the aaptamines.

The cytotoxic alkaloid pigment lihouidine (**16**) was isolated from a crimson new species of *Suberea* (Aplysinellidae, Verongida) sponge, obtained from Lihou Reef (Coral Sea) in 1996.[51] Lihouidine, structure of which was determined by X-ray analysis, contains two modified aaptamine structural units spiro-joined through a six-membered ring and also displays a fused quinoline system. Compound **17**, the pentacyclic core of **16**, has been recently synthesized.[52]

Structural elucidation of the natural products **1** and **3** and confirmation of the skeleton of **3** were carried out by a combination of spectroscopic and chemical techniques. Nakamura et al. submitted **1** to a catalytic hydrogenation over Adams catalyst in a HCl/AcOH mixture, affording the 2,3-dihydrocompound **21**, the acetylation of which with acetic anhydride gave **22a** (Scheme 1). Next, ozonolysis of **22a** furnished the diester **23**, an intermediate in the degradation to keto-ester **24**. Starting with 9-demethylaaptamine (**3**), the same reaction sequence gave diester **23**, establishing that compound **3** was a monodemethyl derivative of **1**. NOE experiments and structural correlation with demethyl(oxy)aaptamine (**6**) also determined the position of the single methoxy group in **3**. During these studies, it was also demonstrated that **6** could be accessed by aerial oxidation of **3**.



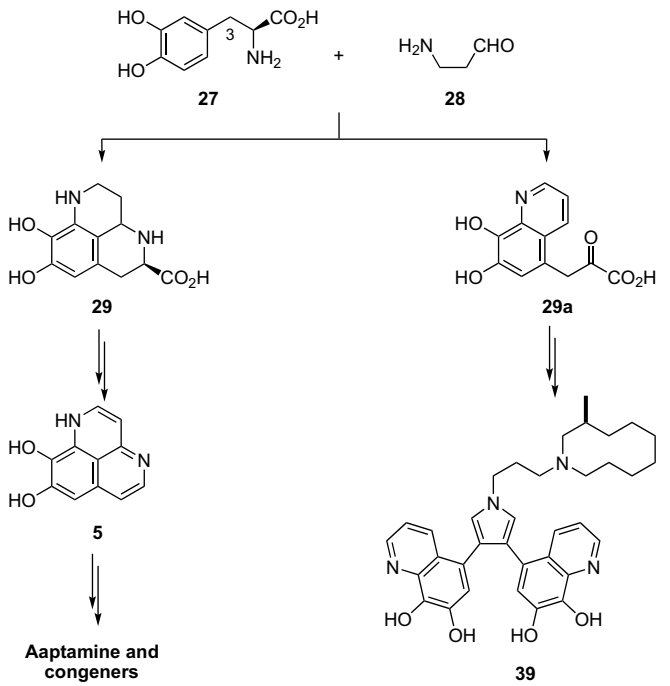

**Scheme 1.** Reagents and conditions: (a) $H_2$, $PtO_2$, HCl/AcOH, overnight (**21**, 70%; **25**, 93%); (b) $Ac_2O$, $C_5H_5N$, 60 °C, 3 h (**22**, 99%; **26**, 81%); (c) $O_3$, MeOH, −78 °C, 1.5 h (**22**→**23**, 62%; **26**→**23**, 38%); (d) 1. $O_3$, MeOH, −78 °C, 4 h; 2. $SMe_2$ (74%); (e) $O_2$ (1 atm), $Na_2CO_3$, MeOH, rt, 1 h (71%).

## 3. Biosynthetic considerations

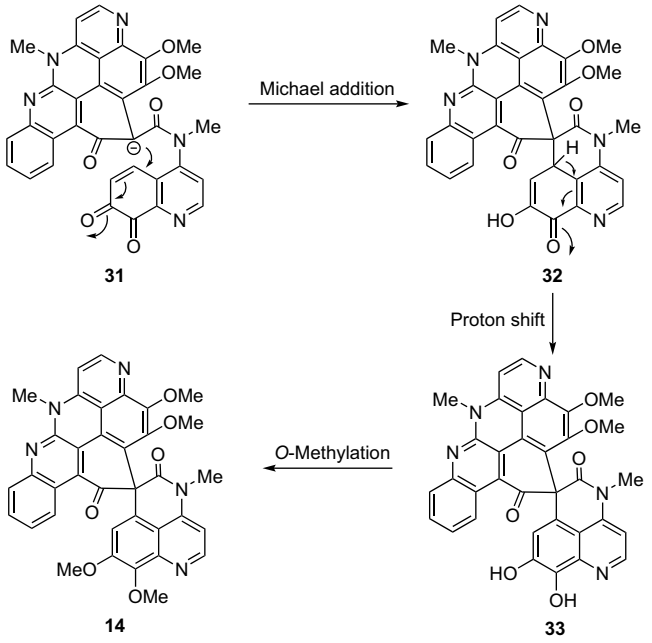
As shown in Scheme 2, the aaptamine skeleton can be assumed to be derived from *S*-3(3,4-dihydroxyphenyl) alanine (L-DOPA, **27**) and a biosynthetic equivalent of β-alanine aldehyde (**28**), which is the formal Michael addition product of acrolein and ammonia. Both compounds are also precursors in Baldwin's hypothesis for the biosynthesis of manzamine alkaloids.[53] Alternatively, aminoaldehyde **28** could be derived from aspartic acid by a decarboxylation/reduction sequence and Joule et al. pointed out to asparagine as the presumable biogenetic precursor.[54]

A biochemical Pictet–Spengler condensation followed by oxidative closure of the piperidine ring C should yield the perhydro derivative **29**.[55a,b] Next, successive decarboxylation and dehydrogenation of **29** would afford 8,9-bisdemethylaaptamine (**5**), from which the other naturally occurring aaptamine alkaloids could be formed by biological methylation. Interestingly, the hypothetical precursor **29a**[12,56] of the cytotoxic bisquinoline pyrrole alkaloid halitulin (**30**), isolated from the sponge *Haliclona tulearensis* (Haplosclerina, Chalinidae) collected in Sodwana Bay (Durban, South Africa),[56] appears to originate from the same two building blocks **27** and **28**. This points out to a common biosynthetic origin of these compounds. Curiously, feeding experiments with tyrosine-3-$^{13}$C demonstrated its incorporation into necatorone (**20a**), suggesting its biogenesis from L-DOPA and anthranilic acid.[55c]

On the other hand (Scheme 3), it was proposed that the last steps of the biogenesis of lihouidine (**16**) presumably involve enolate attack by a β-dicarbonyl intermediate (**31**) in a Michael fashion on an *ortho*-quinonoid ring to produce a spiro ring junction (**32a**)




**Scheme 2.** Proposed biosynthetic pathway for aaptamine and congeners, through **5**, and for halitulin (**30**) through the intermediacy of **29a**.




**Scheme 3.** Proposed last steps of the biosynthetic pathway for the crimson *Suberea* sponge alkaloid lihouidin (**16**).

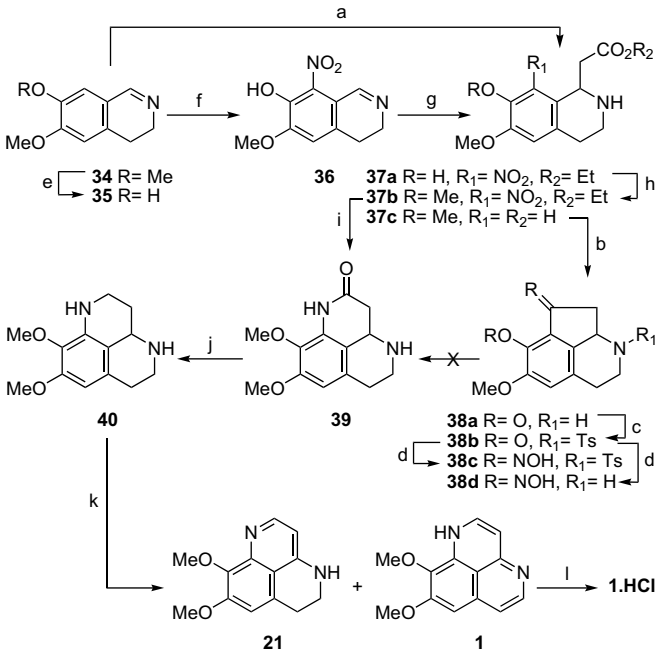
that would be expected to be racemic. Next, a proton shift would result in an *ortho* substituted diphenol (**33**) that could then be bis-*O*-methylated on the functionalized aaptamine moiety that contains the spiro linkage, to afford the natural product **16**.

## 4. Chemical syntheses of aaptamine and aaptaminoids, their analogs and derivatives

The published syntheses of aaptamine use either the isoquinoline (AB) or quinoline (AC) components of the benzo[*de*][1,6]-naphthyridine ring as a platform to build the third nucleus. The various syntheses of the natural product and related compounds will be discussed next, taking into account this difference in the strategic approaches, followed by the synthesis of natural and unnatural related compounds.

### 4.1. Syntheses of aaptamine employing isoquinolines as key intermediates (AB–C)

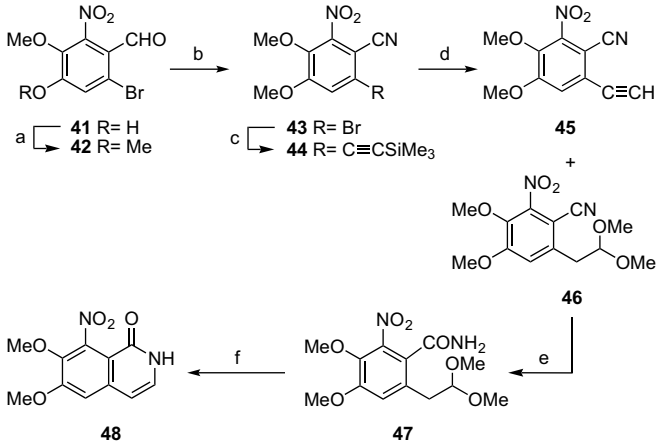
The first total synthesis of aaptamine was described in 1985 by Pelletier and Cava, through the intermediacy of key lactam **39**.[57] Initial attempts, consisting in condensation of dihydroisoquinoline **34**[58] with malonic acid, followed by decarboxylation to acid **37c** and PPA-mediated cyclization to ketone **38a** and sulfonamidation to **38b** were unsuccessful, since none of them could be made to undergo the required Schimdt reaction ($NaN_3$, $H_2SO_4$) to **39**. Moreover, the related oximes **38c**,**d** were unreactive when Beckman rearrangement conditions were applied. Therefore, **34** was selectively demethylated according to Brossi's procedure[59] in order to obtain 64% of the phenolic isoquinoline **35** (Scheme 4). Treatment of **35** with 40% nitric acid and a catalytic amount of $NaNO_2$[60] gave compound **36** in 60% yield, which was converted into nitro ester **37a**[61] (72%) when heated to 120 °C with the monoethyl ester of malonic acid, and further transformed into nitro ester **37b** upon methylation of the phenol moiety with diazomethane, in 96% yield. Hydrogenation of this product afforded the desired isoquinolinolactam **39**, via the corresponding amino ester. Conversion of **39** into aaptamine was then accomplished in three steps, by reduction with diborane–THF complex to 2,3,3a,4,5,6-hexahydroaaptamine (**40**) in



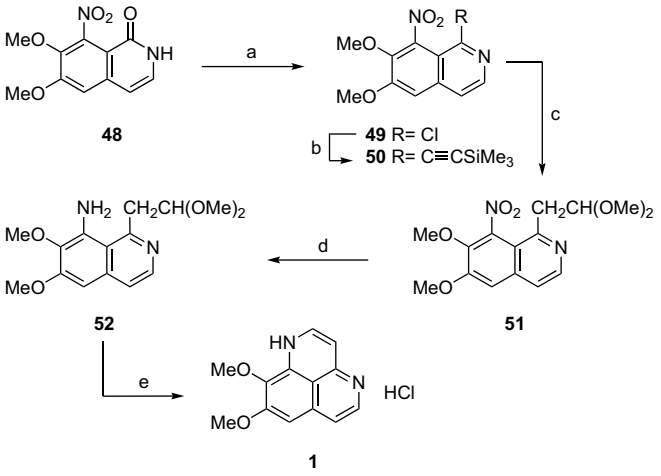

**Scheme 4.** Reagents and conditions: (a) $HO_2CCH_2CO_2H$, 120 °C (72%); (b) PPA, 100 °C (60%); (c) TsCl, $C_5H_5N$ (94%); (d) $NH_2OH{\cdot}HCl$, $C_5H_5N$, EtOH (100%); (e) 48% HBr, 95 °C, 12 h (64%); (f) 40% $HNO_3$, $NaNO_2$ (cat.), 0 °C, 30 min (61%); (g) $HO_2CCH_2CO_2Et$, 120 °C, 60 min (72%); (h) $CH_2N_2$, $Et_2O/CH_2Cl_2$ (96%); (i) $H_2$ (3 atm), 10% Pd/C (cat.), AcOH, 1 h (77%); (j) $B_2H_6$, THF, reflux, 2 h (95%); (k) 1. 5% Pd/C, xylene, reflux, 3.5 h; 2. chromatography on $Al_2O_3$ (**21**, 45%; **1**, 39%); (l) HCl.

95% yield, followed by Pd/C catalyzed dehydrogenation to a roughly 1:l separable mixture of aaptamine free base (**1**) and 5,6-dihydroaaptamine (**21**) in combined 84% yield. Final treatment of **1** with HCl gave the corresponding hydrochloride (**1**·HCl) in 7.9% overall yield.

In 1986, Yamanaka et al.[62] described a five-step synthesis of aaptamine in satisfactory overall yield, starting from 6,7-dimethoxy-8-nitro-1(2*H*)-isoquinolone (**48**) and using palladium-catalyzed reactions as key steps. This was the third total synthesis of the natural product. Different strategies for the synthesis of the required isoquinolone **48** were tested (Scheme 5). In their most efficient procedure, bromo-nitrobenzaldehyde **42**, prepared in 88% yield by O-methylation of 6-bromo-4-hydroxy-3-methoxy-2-nitrobenzaldehyde (**41**),[63] was converted to




**Scheme 5.** Reagents and conditions: (a) MeI, $K_2CO_3$, 50 °C, 5 h (88%); (b) 1. $H_2NOH$, NaOAc, EtOH, reflux, 12 h; 2. $Ac_2O$, reflux, 20 h (96%); (c) TMS–C≡CH, $(Ph_3P)_2Cl_2Pd$, CuI, $Et_3N$, DMF, 45 °C, 1 h (86%); (d) NaOMe, MeOH, DMF, 45 °C, 3 h (**45**, 19%; **46**, 67%); (e) 30% $H_2O_2$, $Na_2CO_3$, 50 °C, 2 h (75%); (f) TsOH, MeOH, PhH, reflux, 12 h (90%).



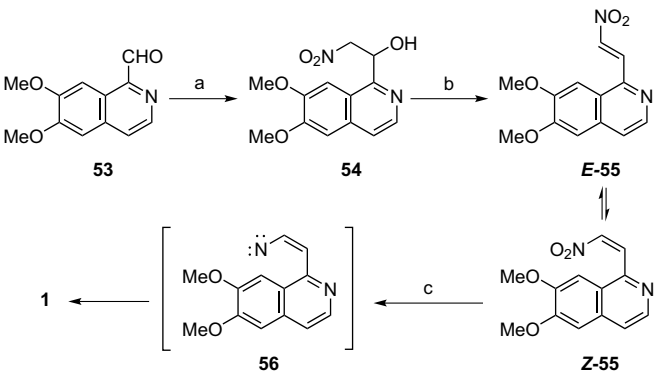

**Scheme 6.** Reagents and conditions: (a) $POCl_3$, reflux, 30 min (92%); (b) TMSA, $(Ph_3P)_2Cl_2Pd$, CuI, $Et_3N$, DMF, 65 °C, 2 h (85%); (c) NaOMe, MeOH, DMF, 60 °C, 1.5 h (63%); (d) $H_2$ (1 atm), 10% Pd/C, MeOH (94%); (e) HCl, MeOH, 1.5 h (45%).

bromo-nitrobenzonitrile **43** (96%) via the corresponding aldoxime. Next, condensation of **43** with trimethylsilylacetylene (TMSA) in the presence of dichlorobis (triphenylphosphine) palladium proceeded smoothly to give 83% of the expected 3,4-dimethoxy-2-nitro-6-(trimethylsilylethenyl) benzonitrile (**44**).

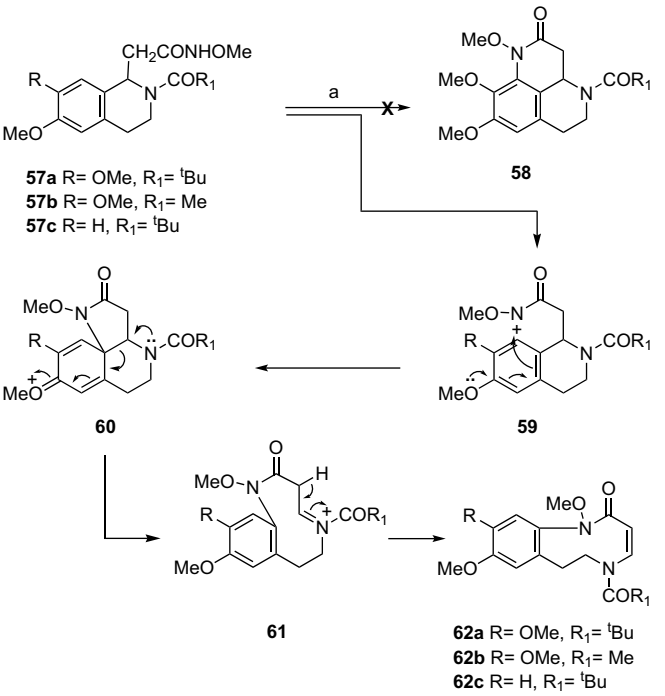
When **44** was treated with sodium methoxide, acetal **46** was obtained in 67% yield, together with small amounts of the desilylated by-product **45** (19%). Finally, partial hydrolysis of the cyano moiety of **46** with alkaline hydrogen peroxide gave benzamide derivative **47** (75%), which on heating with *p*-toluenesulfonic acid, smoothly cyclized to furnish the desired **48** in 90% yield.

The synthesis of aaptamine from **48** (Scheme 6) was carried out employing the authors' previously described method for naphthyridine cyclization.[64] The dehydroxy-chlorination of **48** with phosphoryl chloride under conventional conditions afforded 1-chloroisoquinoline **49** (92%), which, reminiscing the synthesis of **47** from **43**, was allowed to react with TMSA under palladium catalysis, yielding 85% of the silyl acetylene derivative **50**. In turn, nitro-acetylenic isoquinoline derivative **50** was treated with sodium methoxide to afford acetal **51** (63%), which was readily hydrogenated in the presence of palladium on carbon, furnishing 94% of the corresponding aminoacetal **52**. Treatment of the latter with methanolic HCl at room temperature gave aaptamine hydrochloride **1**·HCl, in 22.6% overall yield from intermediate **48**.

In 1987, the group of Tollari[65] disclosed a simple and selective synthesis of aaptamine, through a vinylnitrene intermediate (Scheme 7). To that end, nitromethane was condensed with isoquinoline carboxaldehyde **53**[66] at 0 °C and the resulting




**Scheme 7.** Reagents and conditions: (a) $MeNO_2$, $Et_2NH$, 0 °C, 1 h; (b) $Ac_2O$, $C_5H_5N$, 0 °C, 14 h (85% overall); (c) $P(OEt)_3$, reflux, 150 min (58%).



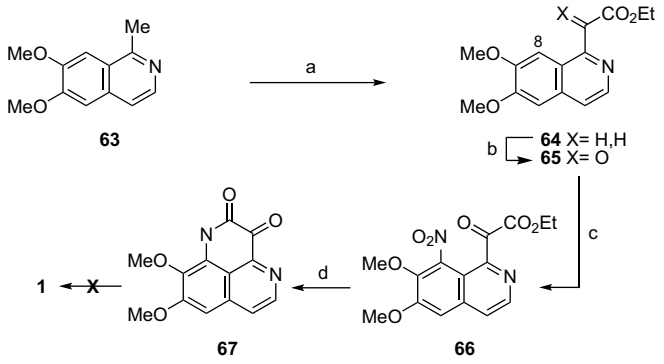

**Scheme 8.** Reagents and conditions: (a) $PhI(OCOCF_3)_2$, 65 °C, 3 min (**62a**, 62%; **62b**, 55%, **62c**, 76%).

nitroalcohol **54** was immediately dehydrated with pyridine and acetic anhydride at 0 °C to give the nitroolefin **55** in 85% overall yield. Submission of the latter to reduction with triethylphosphite[67] under refluxing conditions gave aaptamine in 49.3% overall yield, probably through the intermediacy of **56**. The *Z*-**55** isomer, probably resulting from a thermally induced $E \rightarrow Z$ isomerization of **55**, was considered the likely precursor for the cyclization.

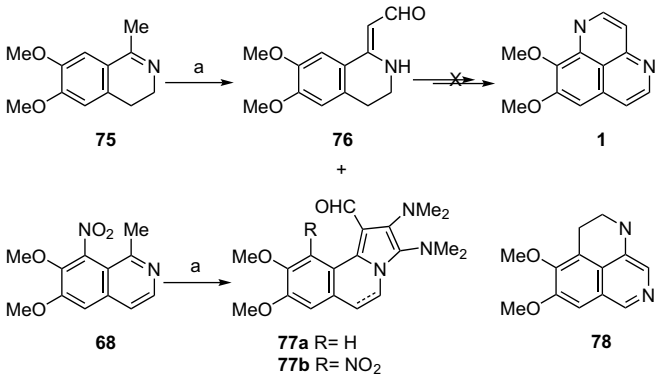
Interestingly, Kikugawa and Kawase also attempted to apply the cyclization of *N*-methoxy-*N*-acylnitreniums to the synthesis of aaptamine.[68] To that end (Scheme 8), tetrahydroisoquinoline derivatives **57a**–**c**[69] were subjected to reaction with $PhI(OCOCF_3)_2$; however, when **57a** was employed as substrate, this resulted in a ring expansion to 1,5-benzodiazonine **62a** in 62% yield,[70] and none of the expected *N*-methoxy lactam **58** was isolated. This was rationalized as being a consequence of the spirocyclization of the *N*-methoxy-*N*-acylnitrenium **59** to form intermediate **60**; fission of the ring junction of the latter would then result in the ring expansion to **61**,[71] which would afford the observed products.

In 1990, Joule et al.[72] reported a series of efforts toward the synthesis of aaptamine. After some unsuccessful attempts, the authors described the synthesis of tricyclic derivative **67** from 1-methylisoquinoline derivative **63**,[73] by a sequence entailing lateral metalation of the heterocycle[74] and reaction of the organometalic species with ethyl chloroformate to yield ester **64** in 21% yield, followed by $SeO_2$-mediated oxidation (89%) to furnish glyoxylate **65** (Scheme 9). This sequence was continued with the highly selective nitration of the isoquinoline nucleus at the position 8 with fuming nitric acid at low temperature, furnishing **66** in 57% yield and spontaneous cyclization to **67** (18%) upon catalytic hydrogenation of the nitro moiety to the related amine.

Unfortunately, however, suitable conditions for changing the oxidation state of the α-ketolactam moiety were not found and the tricycle **67** could not be converted into aaptamine. Despite of the easy availability of the starting material and the small number of synthetic steps involved, the low yields of the cyclization step toward **67** moved these authors to change their strategy leading to the tricycle.

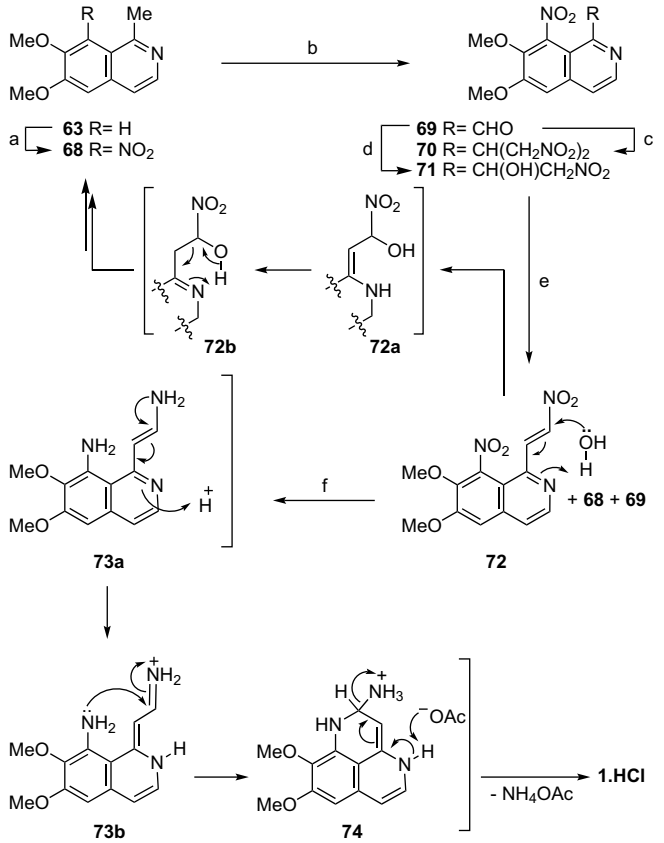



**Scheme 9.** Reagents and conditions: (a) 1. *n*-BuLi, THF, rt, 0.5 h; 2. $ClCO_2Et$, THT, rt, 2 h (21%); (b) $SeO_2$, dioxane, reflux, 1 h (89%); (c) fuming $HNO_3$, −40 °C, 2.5 h (57%); (d) $H_2$ (1 atm), $PtO_2$, EtOH/THF, 1 h (18%).




**Scheme 11.** Reagents and conditions: (a) $POCl_3$, DMF, 0 °C→rt, 2 h.

Therefore, a more efficient approach was pursued, based on previously reported Tollari's strategy for the homologation of the C-1 side chain of the isoquinoline starting material.[65] Coupled to a cyclization reaction under reducing conditions (Scheme 10), this resulted in a five-step total synthesis of aaptamine. Thus, 1-methyl-isoquinoline derivative **63** was selectively nitrated to give 41% of the mononitro derivative **68**,[75] which was oxidized with $SeO_2$ to aldehyde **69** in 54% yield. After testing several bases, it was found that reaction of the aldehyde with basic $Al_2O_3$ in nitromethane gave the trinitro-compound **70**, resulting from Michael addition of nitromethane to the required intermediate **72** (51%). However, changing the proportion of the reactants, it was possible to obtain nitroalcohol **71** in 84% yield.




**Scheme 10.** Reagents and conditions: (a) fuming $HNO_3$, −45 °C, 45 min (41%); (b) $SeO_2$, dioxane, reflux, 2.5 h (54%); (c) $Al_2O_3$ (50-fold excess), $MeNO_2$, reflux, 3.5 h (51%); (d) $Al_2O_3$, $MeNO_2$ (8 equiv), reflux, 3.5 h (84%); (e) $Al_2O_3$, PhH, reflux (**72**, 36%; **68**, 22%; **69**, 19%); (f) 1. $Fe^0$ powder, AcOH, EtOH; 2. HCl (89%).

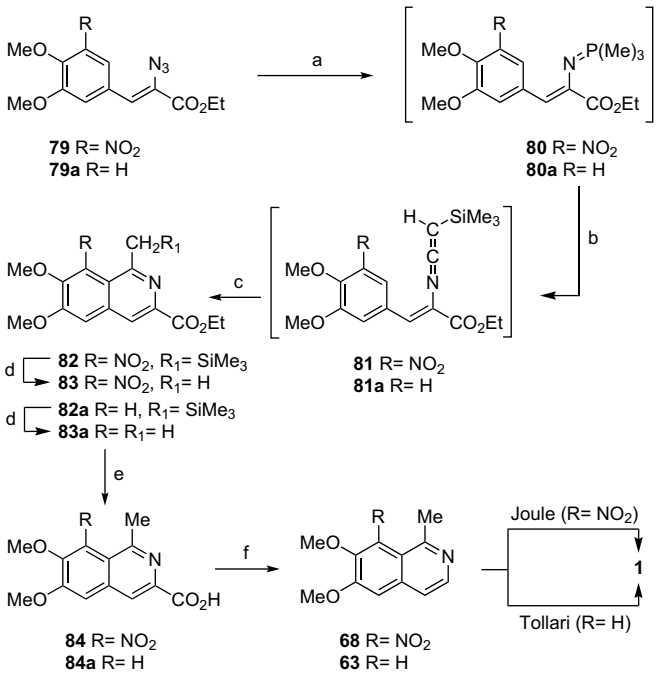
Access to **72** was achieved in 36% yield after refluxing a benzenic solution of **71** over $Al_2O_3$, with azeotropic removal of water; however, this was accompanied by aldehyde **69** and the starting 8-nitroisoquinoline derivative **68**, probably resulting from loss of the elements of nitroformaldehyde through hydration to **72a** and rearrangement via **72b**, in a 1.9:1:1.2 ratio, respectively. Finally, the reductive cyclization of nitroalkene **72** was effected with iron powder in acetic acid,[76] yielding aaptamine hydrochloride in 89% yield after workup and salification. Under these mild acidic conditions, the dinitro-olefin would be reduced to the corresponding amino-enamino isoquinoline **73a**, which in turn may rearrange to the protonated form of the related amino-imino derivative **73b**, cyclizing to aminal **74**; final elimination of ammonia after protonation of the amino-group of the aminal, driven by rearomatization of the isoquinoline moiety, would give rise to the final product, aaptamine.

In 1994, Nagarajan and Rodrigues reported unsuccessful attempts to synthesize aaptamine.[77] Their failed strategy (Scheme 11) started with the Vilsmeier–Haack formylation of the 3,4-dihydroisoquinoline **75**; depending on the reaction conditions this afforded mixtures of acetaldehyde derivative **76** and pyrroloisoquinoline **77a**. In addition, in a related effort and under forcing conditions, the analogous 8-nitroisoquinoline derivative **68** gave **77b**.

In search for aaptamine analogs, the same authors also informed that the cyclization of *N*-benzyl-*N*-hippuryl homoveratrylamine with a mixture of $POCl_3$ and $P_2O_5$ surprisingly gave 3-phenyl-5,6-dihydro-8,9-dimethoxy-imidazo [5,1-*a*]isoquinoline and its 1-benzyl derivative, instead of their expected product, carrying the 2,3-dihydro-1*H*-benzo[*de*][1,7] naphthyridine skeleton **78**.[78]

Later on, in 1996, Molina et al.[79] demonstrated the power of the aza-Wittig strategy for the preparation of two isoquinolines, previously employed as key intermediates toward aaptamine, thus performing formal total syntheses of the natural product. Compound **63** was previously employed in Joule's synthesis[72] and nitroisoquinoline **68** was used in Tollari's sequence.[65] For the synthesis of **68**, vinyl azide **79** was transformed into nitroisoquinoline derivative **83** in 62% yield.

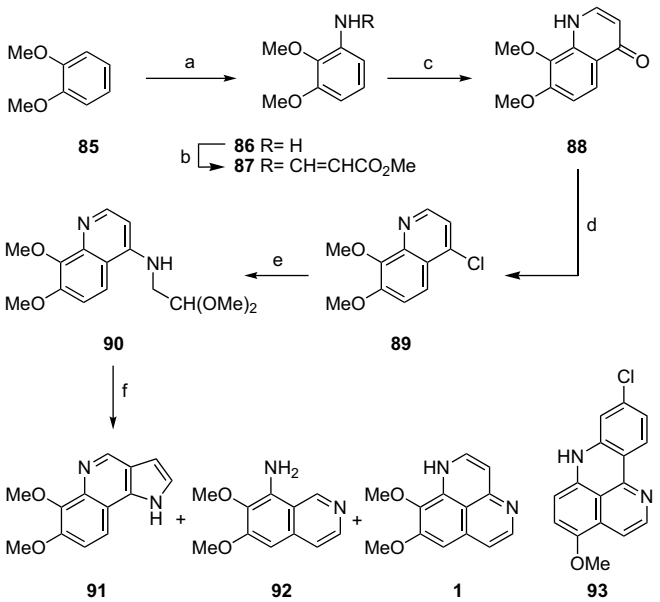
This can be understood as taking place through an initial Staudinger reaction to give iminophosphorane **80**,[80] followed by an aza-Wittig-type reaction with trimethylsilyl ketene to afford silyl ketenimine derivative **81**,[81] an electrocyclic ring closure to **82**, and a final carbon–silicon bond cleavage (Scheme 12). Compound **83** was next converted into the desired nitroisoquinoline **68** in 58% overall yield, by basic hydrolysis to acid **84** and subsequent thermal decarboxylation in diphenyl ether at 180 °C. Analogous isoquinoline derivative **63** was prepared in 50% overall yield from **79a**, employing similar conditions.




**Scheme 12.** Reagents and conditions: (a) $PMe_3$, PhMe; (b) $Me_3SiCH{=}C{=}O$, PhMe; (c) sealed tube, 160 °C, 2 h; (d) $SiO_2$ (**83**, 62%; **83a**, 86%, overall from **79** and **79a**, respectively); (e) LiOH, $THF/H_2O$, rt, 12 h (**84**, 86%; **84a**, 91%); (f) $Ph_2O$, 180 °C 36 h (**68**, 68%; **63**, 64%).

### 4.2. Syntheses of aaptamine employing quinolines as key intermediates (AC–B)

In 1985, Kelly and Maguire[82] disclosed their total synthesis of aaptamine, the first to be carried out through the intermediacy of quinolines (Scheme 13). It comprised six steps, from the easily available veratrole (**85**). In their sequence, *ortho*-metalation of **85** was followed by reaction with TMS/azidomethane to furnish amine **86**,[83] which was subjected to Michael addition[84] with methyl propiolate in methanol, leading to β-aminoacrylate **87**. This set the stage for accessing key quinolone **88**, by thermal cyclization, in refluxing diphenyl ether as high boiling solvent.[84] Next, dehydroxychlorination of **88** with $POCl_3$ gave the 4-chloroquinoline derivative **89**, which once reacted with aminoacetaldehyde dimethyl acetal, afforded the aminoacetalic key intermediate **90**.[85]

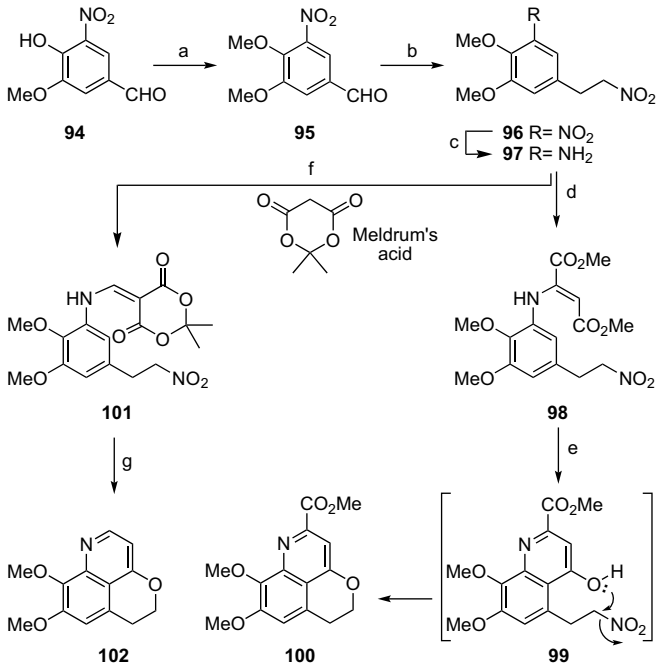



**Scheme 13.** Reagents and conditions: (a) 1. *n*-BuLi, TMEDA, 0 °C; 2. $Me_3SiCH_2N_3$ 3.5 h (78%); b) $HC{\equiv}CO_2Me$, MeOH, 4 days, rt; c) $Ph_2O$, reflux, 25 min (72%, overall); (d) $POCl_3$, rt, 1 day (86%); (e) $H_2NCH_2CH(OMe)_2$, DMSO, 5 days, 95 °C (52%); (f) $F_3CSO_3H$, $SbF_5$, TFA, 75–80 °C (**91**, 33%; **1**, 34%; **92**, 24%).

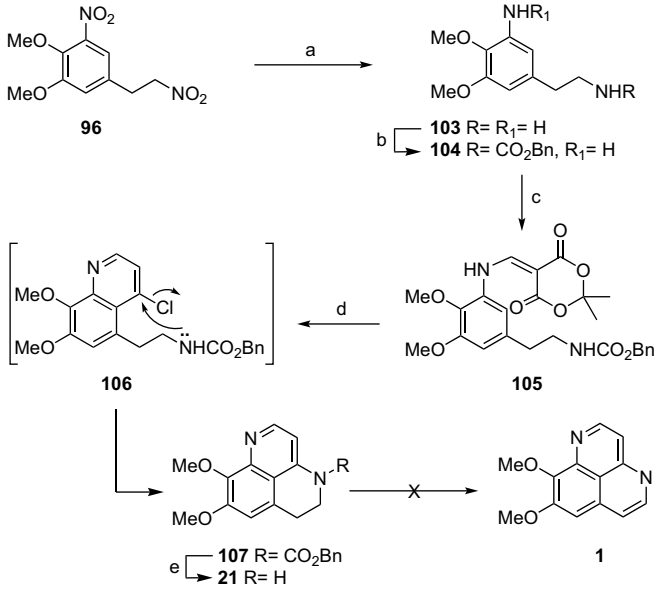
In turn, compound **90** was induced to undergo an intramolecular Pomeranz–Fritsch type[86] reaction, yielding approximately similar amounts of aaptamine (**1**, 8.6% overall yield) and pyrroloquinoline **91** (8.63% overall yield), together with a smaller quantity of anilino-isoquinoline **92**.[87] The best results were achieved when a mixture of triflic acid and antimony pentafluoride in trifluoroacetic acid was used.[88] Interestingly, a similar approach was disclosed by Demeunynck et al. in their synthesis of the pyrido[2,3,4-*m*,*n*]acridine derivative **93** from 6,9-dichloro-2-methoxy acridine. After observing that electrophilic substitution of 2-substituted 9-haloacridines with formaldehyde takes place at the C-1 position, these authors reacted the dihaloacridine with aminoacetal and cyclized the resulting intermediate to **93** with methanesulfonic acid, in 64% yield.[89]

In 1987, Andrew and Raphael[90] unveiled a synthesis of aaptamine characterized by its brevity and the lack of ambiguity in the key cyclization step. In the initially tested route (Scheme 14), the authors converted 5-nitrovanillin (**94**) to 5-nitroveratraldehyde (**95**),[91] which was employed as a substrate to perform a Henry reaction with nitromethane in hot AcOH, under promotion of ammonium acetate. The resulting nitroolefin was selectively reduced with $NaBH_4$ to the saturated nitrocompound **96**, which in turn was hydrogenated over a platinum catalyst, furnishing **97**. Treatment of the latter with dimethyl acetylene dicarboxylate gave the aminoacrylate **98**, which once subjected to cyclization in refluxing diphenyl ether gave only traces of the expected quinolone, the major product being the heterocycle **100**, in 78% yield. This was probably a result of nucleophilic displacement of the nitro group by the intermediate 4-hydroxyquinoline derivative **99**, a transformation recognized as an uncommon event.[92]

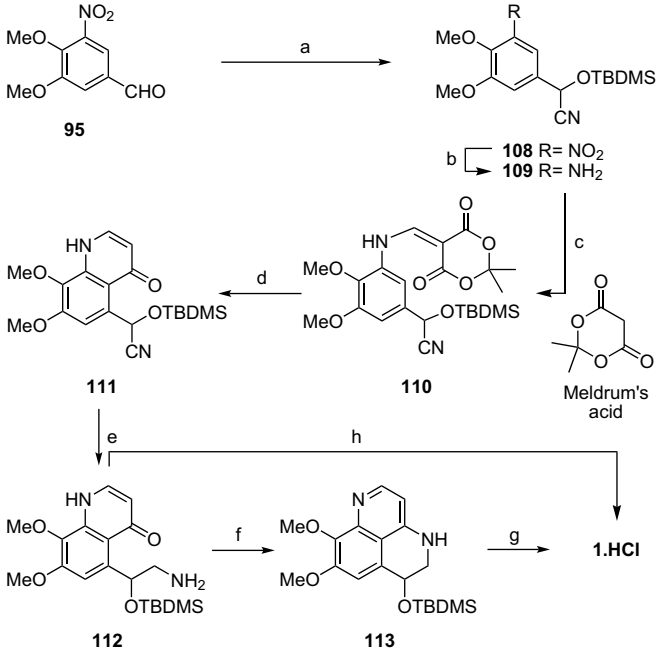
The reaction took the same course, yielding **102**, when compound **101**, the aminomethylene condensation product of **97** with




**Scheme 14.** Reagents and conditions: (a) $Me_2SO_4$, $CH_2Cl_2/H_2O$, $BnN^+Bu_3{\cdot}Br^-$, NaOH, 24 h, rt (81%); (b) $MeNO_2$, $NH_4AcO$, AcOH, 100 °C; 2. $NaBH_4$, MeOH/EtOAc (92%); (c) $H_2$, $PtO_2$, AcOH (95%); (d) $MeO_2CC{\equiv}CCO_2Me$, MeOH, rt, 12 h (77%); (e) $Ph_2O$, reflux, 2 min (78%); (f) Meldrum's acid, $HC(OMe)_3$, reflux, 5 h (91%); (g) $Ph_2O$, reflux, 5 min (66%).




**Scheme 15.** Reagents and conditions: (a) Raney-nickel; (b) BnOCOCl, $K_2CO_3$, $CH_2Cl_2$, 0 °C, 2 h (72%); 2. $NaBH_4$, MeOH/EtOAc (92%); (c) Meldrum's acid, $HC(OMe)_3$, reflux, 5 h (93%); (d) 1. $Ph_2O$, reflux, 4 min; 2. $POCl_3$, 145 °C, 45 min (63%); (e) $H_2$, 10% Pd/C, MeOH, rt, 12 h (84%).




**Scheme 16.** Reagents and conditions: (a) 1. KCN, $ZnI_2$, TBDMSCl, MeCN, 48 h, rt (83%); (b) $H_2$, Raney-Ni, MeOH, rt, 3 h (95%); (c) Meldrum's acid, $HC(OMe)_3$, reflux, 5 h (92%); (d) $Ph_2O$, reflux, 5 min (88%); (e) $H_2$, Raney-Ni, MeOH, rt, 12 h (91%); (f) HMDS, TsOH (53%); (g) HCl; (h) 1. HMDS, TsOH, ))), 15 min; 2. reflux, 15 h; 3. HCl (51%).

2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) and triethyl orthoformate, was subjected to the same reaction conditions.

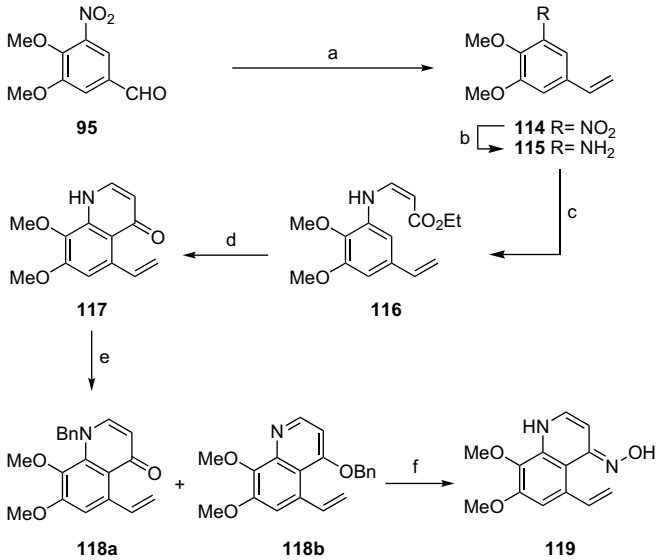
In order to circumvent this problem, **96** was reduced to the diamine **103** (Scheme 15),[93] and this was selectively protected with benzyl chloroformate as the corresponding mono benzylcarbamate **104**. Reaction of the latter with Meldrum's acid and trimethyl orthoformate afforded the corresponding anilinomethylene compound **105**, analogous to **101**, which was thermally cyclized and then treated with phosphorus oxychloride to give the expected tricyclic product **107** through the intermediacy of chloroquinoline **106**. The benzylcarbamate group was then removed by hydrogenolysis, yielding 84% of 5,6-dihydroaaptamine (**21**); however, since **21** proved resistant to dehydrogenation in order to provide aaptamine, this approach was further modified.

Therefore, the authors converted **95** into the silyl-cyanohydrin **108** (Scheme 16) in 83% yield by treatment with potassium cyanide and TBDMSCl.[94] Next, partial hydrogenation of **108** under Raney nickel catalysis gave the aniline **109**, which was smoothly converted into the anilinomethylidene derivative **110** by condensation with Meldrum's acid and trimethyl orthoformate, in 85% yield from **108**.[95] Then, thermal cyclization of **110** with concomitant decarboxylation led to the key quinolone nitrile **111** in 88% yield. Next, selective catalytic hydrogenation of **111** with Raney nickel afforded 91% of the corresponding β-phenethylamine **112**, ready for cyclization.

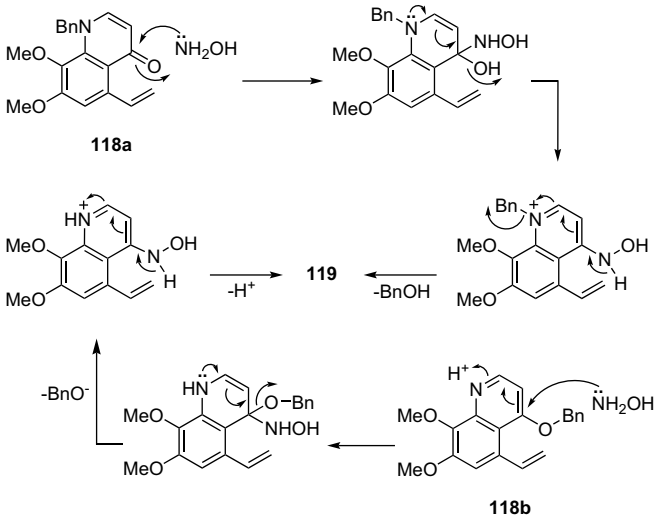
This was effected through a silylation–amination proces with hexamethyldisilazane (HMDS) in the presence of *p*-toluenesulphonic acid as catalyst[96] yielding the air-sensitive tricyclic product **113** (53%). Treatment of the latter with methanolic hydrochloric acid effected its deprotection and the subsequent dehydration, to give aaptamine in 14.4% overall yield. In an improved alternative procedure, the authors used a one-pot reaction to convert **112** directly into aaptamine hydrochloride with the same reagents, thus increasing the overall yield to 24%.

Employing their experience in the synthesis of heteroaromatics by thermal electrocyclic reaction of hexa-1,3,5-trienes[97,98] in 1988, Hibino et al.[99] published their total synthesis of aaptamine, based on the electrocyclization of monoazahexa-1,3,5-triene systems.[100] For the synthesis of key quinolone ring system **117**, the readily available[101] and previously employed 5-nitroveratraldehyde (**95**) was converted in 70% yield into nitrostyrene **114** by a Wittig reaction with methylene triphenylphosphorane (Scheme 17). Next, reduction of the nitro moiety with sodium dithionite provided the aminostyrene **115** (42%), which was treated with ethyl 2-formylacetate[102] to give 62% of the enamino ester **116**.

Preparation of the quinolone nucleus **117** was then achieved in 30% yield by refluxing compound **116** in diphenyl ether. However, attempts to effect the direct transformation of quinolone **117** into the oxime **119** met with failure, presumably because of the vinylogous amide character of the starting material.




**Scheme 17.** Reagents and conditions: (a) $Ph_3P{=}CH_2$, THF, rt, 14 h (70%); (b) $Na_2S_2O_4$, MeOH/$H_2O$, 10 min (42%); (c) $EtO_2CCH_2CHO$, rt, 14 h (62%); (d) $Ph_2O$, reflux, 40 min (30%); (e) 1. NaH, DMF; 2. BnBr, 2 h, rt (**118a**, 33%; **118b**, 33%); (f) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, reflux, 1 h (33% from **117**).




**Scheme 18.** Proposed mechanism for the synthesis of quinolone **119** from **118a** and **118b**.

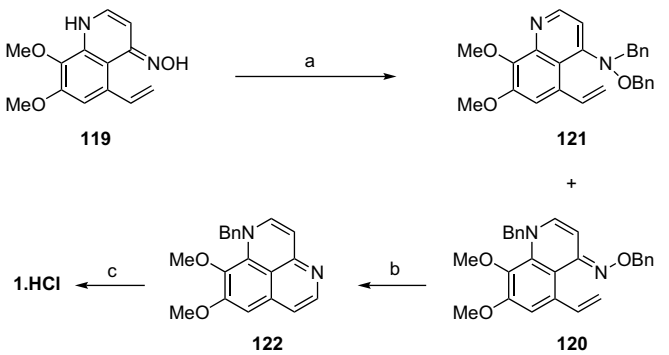
In order to overcome this difficulty, protection of the quinolone nitrogen was suggested. However, attempts of peparing the *N*-benzyl quinolone by reaction with benzyl bromide and sodium hydride in DMF gave a 1:1 inseparable mixture of the *N*- and *O*-protected derivatives **118a** and **118b**.

Fortunately, treatment of the mixture with hydroxylamine hydrochloride gave the debenzylated quinolone oxime **119** in 33% overall yield from **117**. The quinolone oxime **119** could probably arise from both **118a** and **188b**, as shown in Scheme 18.

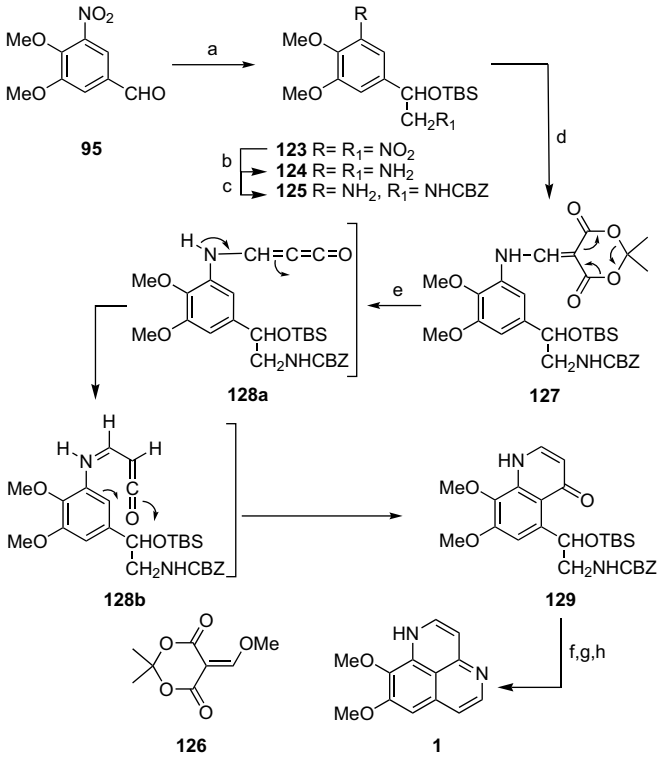
Attempts of a thermal electrocyclic reaction of **119** in refluxing xylene or *ortho*-dichlorobenzene were also unsuccessful, presumably because of the tautomerism between quinolone and quinoline-type forms. Therefore, in order to prevent aromatization, **119** was subjected to benzylation with benzyl bromide; however, this gave an unfavorable 1:1.3 ratio of the *N*,*O*-dibenzylated azahexatriene **120** and the unexpected quinoline **121** in only 30% combined yield (Scheme 19).

Interestingly, however, thermal cyclization of the 1-azahexatriene intermediate **120** in refluxing 1,2-dichlorobenzene afforded 67% of the aaptamine derivative **122**, which resulted in aaptamine hydrochloride **1**·HCl (90%) after final treatment with refluxing concentrated hydrochloric acid.

The hitherto commonly used two-step procedure for the amination of hydroxy *N*-heterocycles by means of treatment with $POCl_3$ with isolation of the corresponding chloro compounds and subsequent amination[103,104] characteristic of the Yamanaka and Kelly syntheses of aaptamine has quite a number of drawbacks;




**Scheme 19.** Reagents and conditions: (a) 1. NaH, DMF; 2. BnBr, DMF, rt, 1 h (**121**, 17%; **120**, 13%); (b) 1,2-$Cl_2$–$C_6H_4$, reflux, 2 h (67%); (c) 12 N HCl, reflux, 2 h (90%).




**Scheme 20.** Reagents and conditions: (a) 1. $MeNO_2$, Amberlyst A-21, rt, 3 h; 2. TBSOTf, 2,6-lutidine, 0 °C→rt (93%); (b) 10% Pd/C, $HCO_2NH_4$, reflux 2 h (100%); (c) CBZCl, DMAP, $K_2CO_3$, −78 °C to −30 °C, 8 h (97%); (d) **125**, reflux, 4 h (86%); (e) $Ph_2O$, 240 °C, 20 min (82%); (f) 10% Pd/C, $HCO_2NH_4$, MeOH, 40 min; (g) $(NH_4)_2SO_4$, $Et_3N$, HMDS, reflux, 20 h; (h) MeOH, HCl, rt, 20 h (85%).

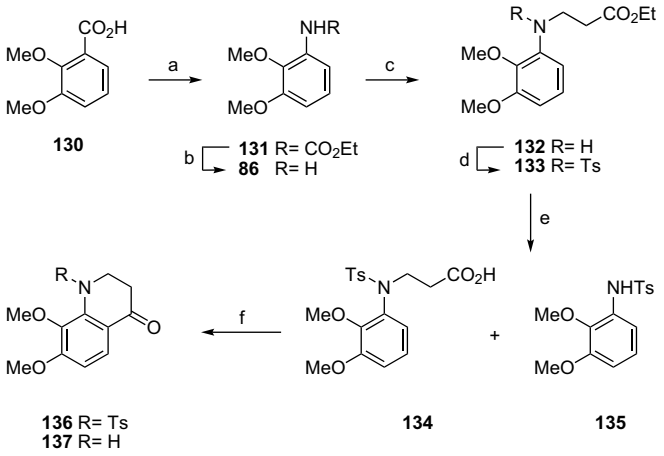
these include the need of appropriate protection of potentially reactive groups in the heterocyclic or amine moieties and the frequently observed chlorination of alkyl groups associated to the hydroxy *N*-heterocycle.

As an improved alternative, in 2000 Walz and Sundberg suggested that the one-pot silylation–amination described by Vorbrüggen et al.[96c] could be used for the synthesis of aaptamine (**1**) from **129** employing hexamethyldisilazane and an acidic catalyst. After a brief exploration of the concept, these authors[105] adapted the Andrew–Raphael route[90] and reported a synthesis of aaptamine in which the key step was the cyclization of a 5-(2-aminoethyl)-4-quinolone derivative to the corresponding benzo-[*de*][1,6] naphthyridine ring, by heating with HMDS and ammonium sulfate.

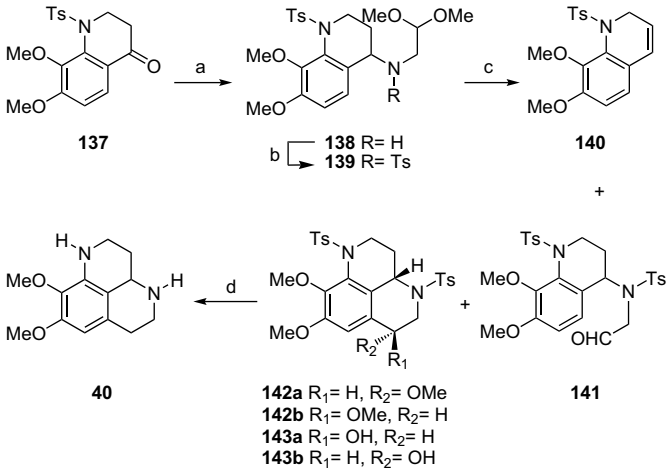
Their sequence toward the quinoline intermediate followed the Conrad–Limpach protocol,[106] starting with the previously used 5-nitroveratraldehyde (**95**),[91] which was subjected to a nitroaldol condensation with nitromethane, under Amberlyst A-21 promotion (Scheme 20).

The resulting nitroalcohol adduct was silylated to afford **123** using TBDMSTf and lutidine,[107] and then subjected to a transfer hydrogenation with ammonium formate and Pd/C, to give the bis-amino intermediate **124** in 93% overall yield.[108] Selective carboxybenzylation of the latter to **125**, followed by condensation with methoxymethylene Meldrum's acid derivative **126**, and cyclization of the resulting **127** furnished dihydroquinolone **129** in 68% yield.

Reminiscing Raphael's aaptamine synthesis, thermal cyclization of **127**, which was carried out in refluxing diphenyl ether, probably took place through the initial decarboxylation of the substrate to give intermediate **128a**, which rearranged to ketene-imine **128b** through a [1,3] hydrogen shift,[109] yielding **129** after electrocyclization. Hydrogenolytic removal of the CBZ group followed by HMDS-mediated cyclization proceeded without inconvenience to give **1**, which was isolated as the corresponding hydrochloride, in




**Scheme 21.** Reagents and conditions: (a) $(PhO)_2P(O)N_3$, EtOH, THF, 65 °C, 2 h (90%); (b) KOH, EtOH, reflux, overnight (97%); (c) $H_2C{=}CHCO_2Et$, AcOH (cat.), reflux, 24 h (84%); (d) TsCl, *i*-$Pr_2NEt$, $CHCl_3$, reflux, 14 h (92%); (e) 1. 10% LiOH, EtOH/$H_2O$, reflux, 24 h; 2. HCl, pH=3 (74%); (f) PPE, PhMe, 55 °C, 2 h (95%).




**Scheme 22.** Reagents and conditions: (a) 1. $H_2NCH_2CH(OMe)_2$, AcOH, $MgSO_4$, 4 Å MS, EtOH, overnight; 2. $NaCNBH_3$, reflux, 24 h (87%); (b) TsCl, *i*-$Pr_2NEt$, $CHCl_3$, reflux, 14 h (64%); (c) $SnCl_4$, $CH_2Cl_2$, −78 °C, 1.5 h, −60 °C overnight (**140**, 23%; **141**, 4%; **142a**, 9%; **142b**, 24%; **143a**+**143b**, 9%); (d) 1. Na, $NH_3$, −33 °C; 2. $NH_4Cl$ (83%).

85% yield. A melting point significantly higher than previously reported was observed (176–177 °C); however, it remained unclear whether this represents a different crystal form or a different state of hydration of the product.

Finally, in 2008, we[110] reported the synthesis of 2,3,3a,4,5,6-hexahydroaaptamine (**40**) in eight steps and 11% overall yield from aniline derivative **132** (Scheme 21). As **39** is the penultimate intermediate of Pelletier and Cava's synthesis,[57a] this strategy constitutes a formal total synthesis of aaptamine (**1**). Whereas access to **40** by the latter authors was achieved employing an isoquinoline derivative as starting material, thus involving an AB-C strategy, this alternative sequence afforded the aaptamine precursor by means of an AC–B ring forming strategy, through the intermediacy of quinoline derivative **137**.

The synthesis started with the preparation of aniline **132**, which was accomplished in 90% yield through the Curtius–Yamada rearrangement[111] of commercially available 2,3-dimethoxybenzoic acid (**130**) with diphenylphosphoryl azide in refluxing EtOH, followed by basic hydrolysis of the intermediate ethyl carbamate **131** (Scheme 21).[52] Next, **86** was submitted to an aza-Michael addition by reaction with refluxing ethyl acrylate under acetic acid promotion, furnishing 84% of the β-amino ester **132**.

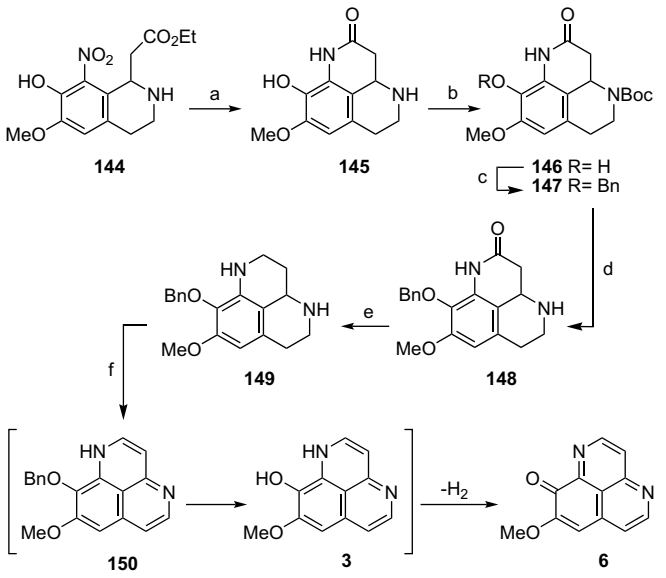
Compound **132** was uneventfully converted into the related sulfonamido derivative **133** by reaction with tosyl chloride and DIPEA in refluxing chloroform (92%). However, attempts to cyclize the sulfonamido ester employing either $SnCl_4$, polyphosphoric ester (PPE) or polyphosphoric acid (PPA)[112] met with failure. Therefore, the ester was subjected to basic hydrolysis to obtain the sulfonamido acid **134**. Not unexpectedly, important quantities of the known sulfonamide **135**,[113a] resulting from the retro-Michael reaction of **133**, were isolated irrespective of the nature of the base employed; however, the use of LiOH in THF/$H_2O$ proved to be the best conditions, furnishing a 3.8:1 mixture of **134** (74% yield) and **135**.

After systematically studying the cyclization of acid **134**, it was found that employing $SnCl_4$ in $CH_2Cl_2$ at 0 °C gave mostly **137**,[113b] while using excess PPE in toluene at 55 °C provided a smooth and clean access to **136** in 95% yield, allowing to undertake the formation of the third ring, as shown in Scheme 22. To that end, quinolone derivative **136** was subjected to a reductive amination reaction with aminoacetal employing sodium cyanoborohydride as selective reducing agent.[114] It was observed that product yields increased when the carbonyl compound was left to react overnight with the amine in the presence of activated 4 Å molecular sieves before adding the reducing agent; this strategy furnished secondary amine **138** in 87% yield. Amine **138** was then submitted to sulfonamidation with tosyl chloride and DIPEA under forcing conditions, yielding 64% of sulfonamidoacetal **139** and setting the stage for the cyclization step.

Use of $SnCl_4$ at −60 °C provided a mixture of methyl ethers **142a**,**b** and alcohols **143a**,**b** in 42% combined yield, accompanied by 23% of compound **140** and 4% of aldehyde **141**.[115] However, submission of the mixture of alcohols and methyl ethers **142** and **143** to reaction with sodium in liquid ammonia effected the simultaneous removal of the tosyl protecting group and the benzylic oxygenated moieties,[116] furnishing the expected 2,3,3a,4,5,6-hexahydroaaptamine (**38**) in 83% yield, which completed the formal synthesis of the natural product.

### 4.3. Synthesis of natural aaptaminoids

Pelletier and Cava[57a] employed the same conceptual strategy that led to aaptamine for the synthesis of demethyl(oxy)aaptamine



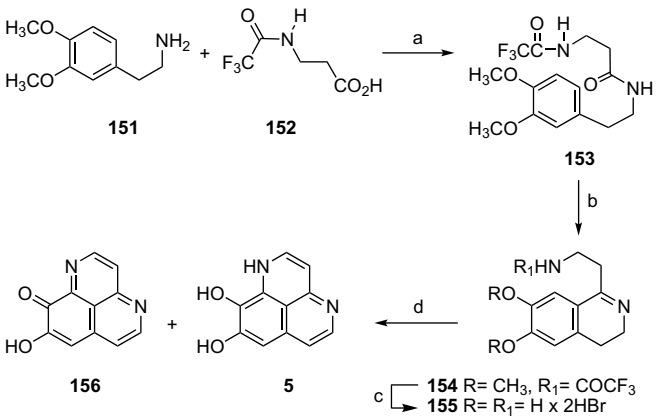

**Scheme 23.** Reagents and conditions: (a) $H_2$, 5% Pd/C, AcOH (100%); (b) $(Boc)_2O$, $CHCl_3$, reflux (88%); (c) BnBr, $K_2CO_3$, acetone, reflux (86%); (d) TFA/$H_2O$ (3:1), 25 °C (96%); (e) $B_2H_6$, THF, 25 °C (69%); (f) 5% Pd/C, xylene, reflux (35%).

(**6**). This started with the catalytic hydrogenation of the nitro ester **144** with 5% Pd/C to quantitatively afford the amino lactam **145** (Scheme 23). Attempts to reduce the lactam with $LiAlH_4$ or diborane were unsatisfactory, the free phenol being suspected as responsible for this outcome; therefore, the phenolic function was protected in a three-stage sequence. First, the corresponding *N*-Boc derivative **146**[117] was formed (88%), which was immediately converted into the benzyl ether **147** in 86% yield; finally, the *N*-Boc group[118] was removed, affording 96% of **148**.

Diborane reduction of lactam **148** was achieved under mild conditions to give 69% of intermediate **149**. In turn, this was refluxed in xylene with a catalytic amount of Pd/C providing demethyl(oxy)aaptamine (**6**) in 17.5% overall yield, through the intermediacy of **150** and 9-demethylaaptamine (**3**), together with a complex mixture of unknown compounds. This dehydrogenation was thought to take place in three steps, including the loss of 3 equiv of hydrogen to yield intermediate **150**, then hydrogenolysis of the benzyl group to give 9-demethylaaptamine (**3**) and finally loss of another mole of hydrogen to furnish **6**.

Steglich et al.[119] reported a concise four-step synthesis of 8,9-bisdemethylaaptamine (**5**) from homoveratrylamine (**151**) and *N*-trifluoroacetyl-β-alanine (**152**), based on biosynthetic considerations (Scheme 24).[120] DCC-mediated coupling of the two starting materials (63%), followed by Bischler–Napieralski cyclization of the resulting amide **153** afforded the 3,4-dihydroisoquinoline **154** in high yield, whose structure was confirmed by X-ray crystallography. On treatment with aqueous hydrobromic acid, **154** underwent cleavage of the methyl ether groups and the more labile trifluoroacetamido moiety, affording 70% of the corresponding catecholamine, isolated as its stable and analytically pure bishydrobromide **155**.

At this point, the envisaged biomimetic key step was carried out, employing an oxidative cyclization of **155** with 1% aqueous KOH and potassium peroxodisulfate or air. Bisdemethylaaptamine (**5**) was the major product obtained (20–49%), accompanied by minor amounts of the unnatural bisdemethyl(oxy)aaptamine (**156**), resulting from further oxidation of **5**. Due to the instability of the free base, **5** was purified as trifluoroacetate or hydrochloride by gel chromatography, affording the product in 20% overall yield. Interestingly, however, experiments to convert the dihydroxy compound **5** or its salts into other natural products, including aaptamine (**1**), isoaaptamine (**2**), 9-demethylaaptamine (**3**) or demethyl(oxy)aaptamine (**6**) by selective methylation were reported to be unsuccessful.[119] A Bischler–Napieralski based synthesis of necatorone (**20a**) was earlier reported by the same group.[55b]




**Scheme 24.** Reagents and conditions: (a) DCC, HOBt, *N*-ethyl morpholine, THF (63%); (b) $POCl_3$, $CH_3CN$, reflux, 8 h (94%); (c) 48% HBr 145 °C, 4–8 h (70%); (d) 1% KOH, $K_2S_2O_8$ (3 equiv), 2 h or air/$O_2$ 1% KOH, 4 h (6%).

After adapting the Andrew–Raphael route,[90] in addition to aaptamine, Walz and Sundberg[105] successfully synthesized isoaaptamine (**2**) and related compounds, including 9-demethylaaptamine (**3**), demethyl(oxy)-aaptamine (**6**), and unnatural 1-methyl-8-demethylaaptamine (**160**) and 8-demethylaaptamine (**161**).

The synthesis of **2** started with the nitroaldol condensation between benzyl ether **157a** and nitromethane, catalyzed by the ion exchange resin Amberlyst A-21 (Scheme 25);[121] quinolone **158a** was obtained after following the general sequence of reactions detailed in Scheme 20. Interestingly, both nitro groups were simultaneously reduced using the $NiCl_2/NaBH_4$[122] reagent giving a primary alkylamine, which was selectively protected with CBZ-Cl employing temperature control, and an arylamine moiety, which was employed for installation of the side chain leading to the said quinolone.

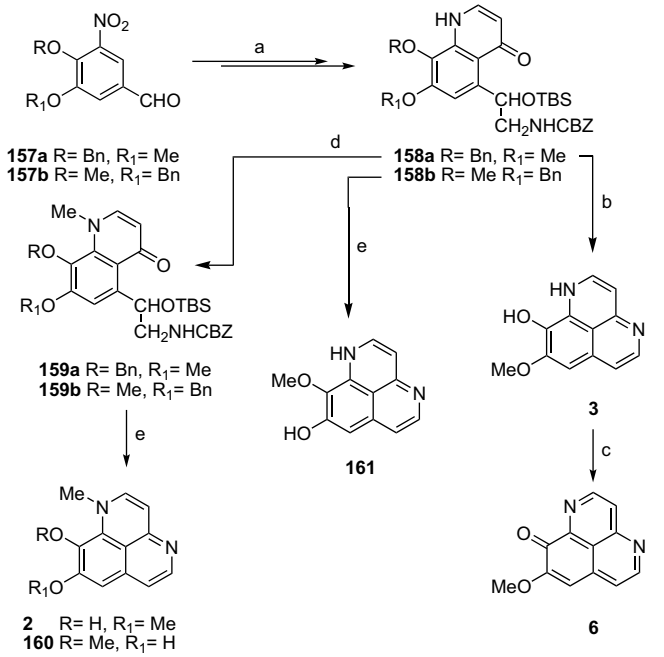
Good regioselectivity for N- versus O-alkylation of the quinolone **158a** was achieved with methyl iodide and $K_2CO_3$ in DMF, yielding **159a**. Removal of the CBZ and benzyl protecting groups by transfer hydrogenolysis,[108] followed by cyclization and aromatization gave **2** in 17% overall yield.

On the other hand, the unmethylated quinolone **158a** ultimately led to 9-demethylaaptamine (**3**), air oxidation of which resulted in demethyl(oxy)aaptamine **6**. Analogously, starting from aldehyde **157b**, and through the intermediacy of quinolones **158b** and **159b**, the unnatural 1-methyl-8-demethylaaptamine (**160**) was obtained in 30% overall yield. On the other hand, the unmethylated quinolone **158b** was converted into the unnatural 8-demethylaaptamine (**161**) in 68% yield.

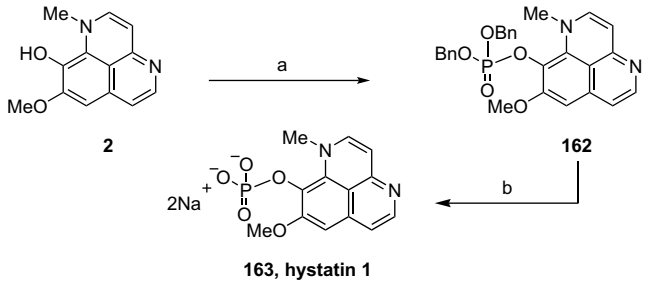
## 4.4. Prodrugs and semisynthetic aaptaminoids

### *4.4.1. Hystatin 1 as a prodrug derived from aaptamine*

When recently isolated, isoaaptamine (**2**) is a yellowish powder that easily darkens, presumably due to air oxidation. Therefore, Pettit et al.[42] directed their attention at the preparation of stable prodrugs that would retain the biological activity of isoaaptamine.




**Scheme 25.** Reagents and conditions: (a) see Scheme 20; (b) 1. 10% Pd/C, $NH_4OAc$, MeOH, 40 °C, 20 min; 2. $(NH_4)_2SO_4$, $Et_3N$, HMDS, reflux, 20 h; 3. MeOH, HCl, rt, 20 h (**3**, 83%); (c) MeOH, $O_2$, 0.2 M $NaHCO_3$, 1 h (73%); (d) $K_2CO_3$, MeI, DMF, 100 °C, 2 h (**159a**, 79%; **159b**, 96%); (e) 1. 10% Pd/C, $HCO_2NH_4$, MeOH; 2. $(NH_4)_2SO_4$, $Et_3N$, HMDS, reflux 20 h; 3. MeOH, HCl, rt, 20 h (**2**, 73%; **160**, 68%; **161**, 76%).




**Scheme 26.** Reagents and conditions: (a) $(BnO)_2(O)PH$, *i*-$Pr_2EtN$, DMAP, $CCl_4$, −10 °C, 2 h; (b) 1. $Me_3SiBr$, $CH_2Cl_2$, rt, 2 h; 2. NaOMe, MeOH, 12 h (45% overall).

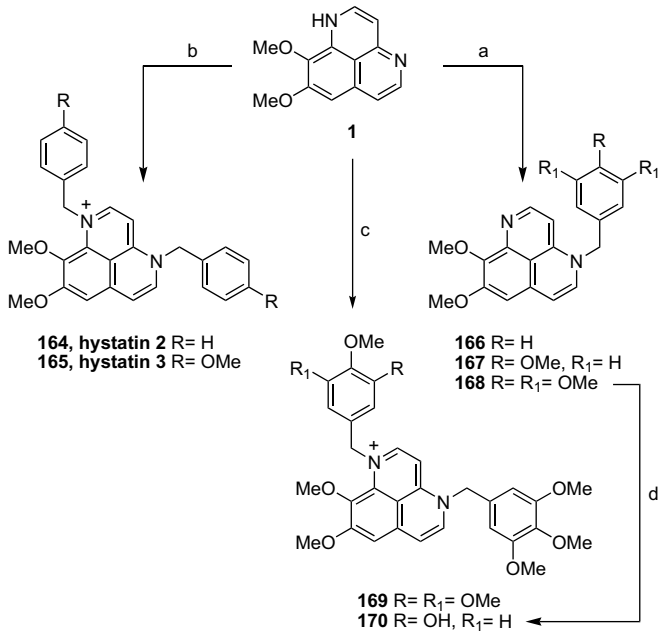
For that purpose, **2** was first phosphorylated[123] with dibenzyl phosphite (Scheme 26) and the resulting intermediate **162** was subjected to cleavage of the benzyl ester by means of trimethylsilyl bromide;[124] reaction of the resulting phosphoric acid with sodium methoxide afforded the relatively more stable disodium phosphate prodrug designated as Hystatin 1 (**163**) in 45% overall yield.

*4.4.2. Semisynthetic N-benzyl derivatives*

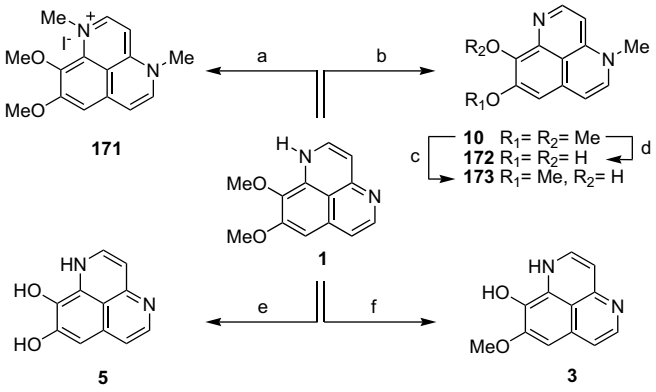
The group of Pettit also prepared the $N_1$,$N_4$-bisbenzyl aaptamine derivatives Hystatin 2 (**164**) and Hystatin 3 (**165**) from aaptamine (**1**), by reaction with benzyl bromide and 4-methoxybenzyl bromide, respectively (Scheme 27).[125]

When sodium hexamethyldisilazane was employed as a base, the 4-*N*-benzyl derivatives **166** and **167** were obtained, in 59% and 65% yield, respectively; however, when the reaction was performed in DMF, using potassium carbonate as base, the quaternary, bisbenzyl derivatives **164** and **165** were accessed in 68% and 89% yields, respectively. The structures of these derivatives were established by X-ray crystallography.

Analogously, the $N_4$-3,4,5-trimethoxybenzyl derivative (**168**, 60%), as well as the $N_1$,$N_4$-bis-3,4,5-trimethoxybenzyl analog (**169**, 89%) and the $N_1$-3-hydroxy-4-methoxy-$N_4$-3,4,5-trimethoxybenzyl heterocycle (**170**, 79%) were synthesized,[125] and their structures were confirmed by X-ray analysis.




**Scheme 27.** Reagents and conditions: (a) $ArCH_2Br$, $K_2CO_3$, DMF, rt, 12 h (**166**, 68%; **167**, 89%; **168**, 59%; (b) $ArCH_2Br$, NaHMDS, THF, −78 °C (**164**, 59%; **165**, 65%); (c) 3,4,5-$(MeO)_3$–$C_6H_2CH_2Br$, NaHMDS, THF, −78 °C (**169**, 89%); (d) 3-OH, 4-MeO–$C_6H_3CH_2Br$, $K_2CO_3$, DMF, rt, 12 h (**170**, 73%).




**Scheme 28.** Reagents and conditions: (a) MeI, $K_2CO_3$ DMF, rt, 12 h (82%); (b) NaHMDS, MeI, −78 °C, 3 h (77%); (c) HBr, 115–120 °C, 1 h (82%); (d) 48% HBr, 150 °C, 6 h (75%); (e) HBr, 145–150 °C, 4 h (77%); (f) HBr, 115–120 °C, 45 min (64%).

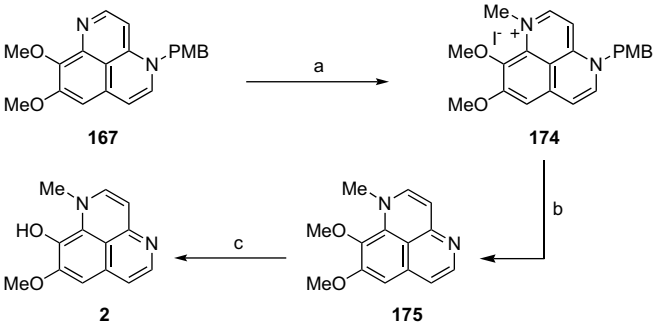
*4.4.3. Semisynthetic O- and N-methyl derivatives*

Aaptamine bears two nitrogens and two oxygens that could be partially methylated, potentially leading to 16 possible derivatives, most of them unnatural. Pettit et al.[126] synthesized half of these 16 possible methyl-derivatives in order to explore their anticancer structure–activity relationships.

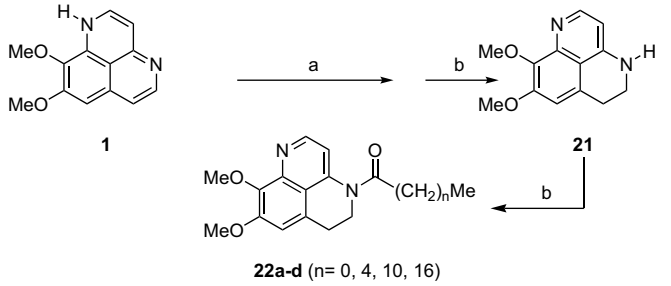
Reaction of aaptamine (**1**) with excess methyl iodide under potassium carbonate promotion led to formation of the permethylated quaternary ammonium derivative **171** (82%). Such quaternary quinolinium alkaloids[127] are not unusual in nature and they often show interesting biological activities (Scheme 28).[128] The selective mono-methylation of aaptamine at the isoquinolinic nitrogen yielding 4-methylaaptamine (**10**) was achieved in 77% yield with methyl iodide, using sodium hexamethyldisilazide (NaHMDS) as base. When this was treated with 48% HBr at 150 °C bis-O-demethylation took place, leading to 75% of the catechol derivative **172**.

The same protocol was applied to aaptamine, furnishing 77% of catechol **5**.[119] However, selective O-demethylation of dimethyl ether **10** at position C-9 could be achieved with 48% HBr at 115 °C, giving **173**, a structural isomer of isoaaptamine (**2**) in 82% yield. When similar conditions were applied to aaptamine, compound **3** was obtained in 64%, the structure of which was confirmed by X-ray crystallography.

Preparation of 1-methylaaptamine derivatives elicited some attention and was also accomplished. The group of Pettit reported the synthesis of isoaaptamine (**2**) from aaptamine,[126] through the intermediacy of 1-*N*-methylaaptamine (**175**). After a series of unsuccessful trials, the 4-*N*-PMB-protected aaptamine derivative **167**, prepared by treatment of aaptamine with NaHMDS and 4-methoxybenzyl bromide (Scheme 29), was reacted with excess of methyl iodide to give 94% of the desired *N*-1 methylated product **174**, the structure of which, indicating that the methyl group was




**Scheme 29.** Reagents and conditions: (a) MeI, $K_2CO_3$, DMF, rt, 12 h (94%); (b) TfOH, TFA, 75 °C, 1 h (70%); (c) 48% HBr, 115–120 °C, 1 h (81%).




**Scheme 30.** Reagents and conditions: (a) $H_2$ (1 atm), $PtO_2$, AcOH/35% HCl, 80 °C, 24 h (60%); (b) $Me(CH_2)_nCOCl$ or $[Me(CH_2)_nCO]_2O$, $C_5H_5N$, rt, overnight (31–59%).

attached to the quaternary nitrogen atom, was confirmed by X-ray crystallography.

The removal of the PMB group also proved problematic, since treatment with TFA, CAN or 48% HBr was unsuccessful. However, reaction of **174** with a mixture of TFA and trifluoromethanesulfonic acid[129] successfully lead to **175** in 70% yield. The selective O-demethylation of dimethyl ether **175** with 48% HBr at 115 °C gave 81% of isoaaptamine (**2**), isolated as the corresponding hydrobromide salt.

*4.4.4. Semisynthetic ester, amide, and dimeric derivatives*

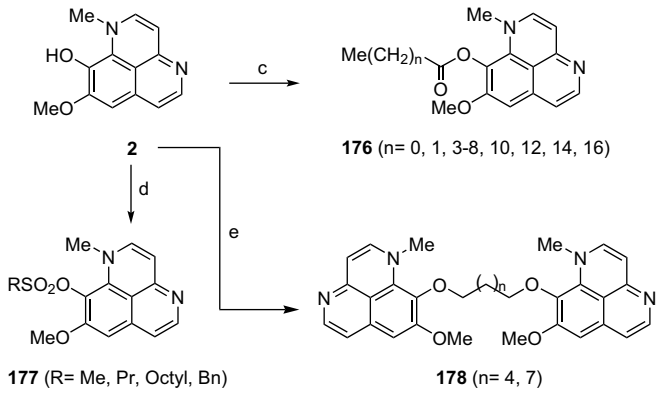
As part of a structure–cytotoxicity relationship study of isoaaptamine, aaptamine and their derivatives, Shen et al.[130] prepared a series of homologous amides (**22a**–**d**) of 2,3-dihydroaaptamine (**21**) in 31–59% yield. The starting amine **21** was synthesized by catalytic hydrogenation of aaptamine (Scheme 30).

In addition, isoaaptamine (**2**) was subjected to esterification with sulfonyl halides, acyl halides, and acyl anhydrides, furnishing 50–94% yield of the homologous straight chain aliphatic ($C_2$–$C_{18}$) esters **176**, as well as sulfonates **177** (Scheme 31), and also dimers **178**, obtained through a Williamson-type etherification.[131] On the other hand and employing the same strategy, for a biological activity study, Gul et al.[132] prepared the *N*,*N*-diethyl and *N*-pyrrolidyl carbamates of isoaaptamine in over 80% yield, along with a series of aryl, aralkyl, haloar(alk)yl, and other functionalized esters.

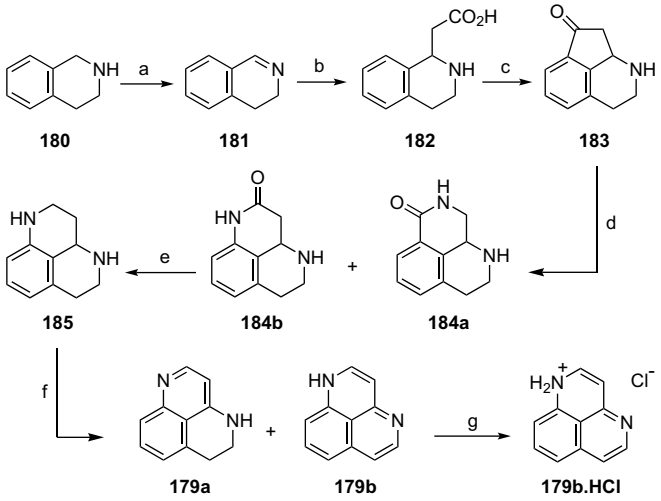
## 4.5. Synthetic, non-natural aaptaminoids

*4.5.1. Synthesis of 1H-benzo[de][1,6]naphthyridine (didemethoxyaaptamine)*

Pelletier and Cava[57a] disclosed the details of successful synthetic approaches to the 1*H*-benzo[*de*][1,6]naphthyridine skeleton (**179b**), the parent structure of the aaptamines.[133]



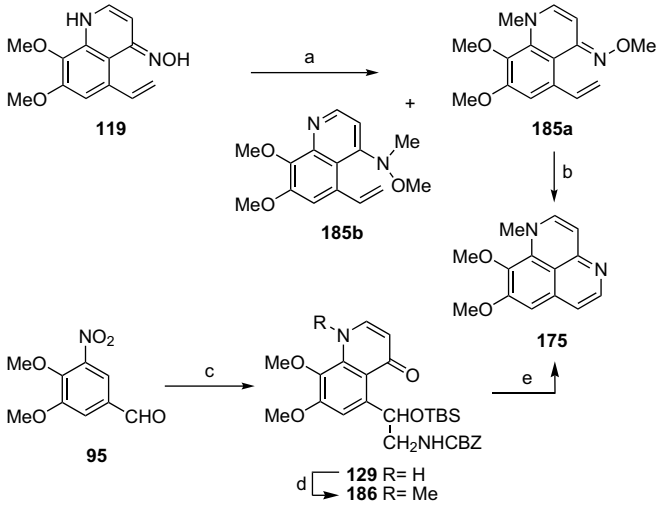

**Scheme 31.** Reagents and conditions: (a) $Me(CH_2)_nCOCl$ or $[Me(CH_2)_nCO]_2O$, $C_5H_5N$, rt, overnight (50–94%); (c) $RSO_2Cl$, $Et_3N$, $CH_2Cl_2$, 0 °C (46–80%); (d) $BrCH_2(CH_2)_nCH_2Br$, $Cs_2CO_3$, $Me_2CO$, reflux.



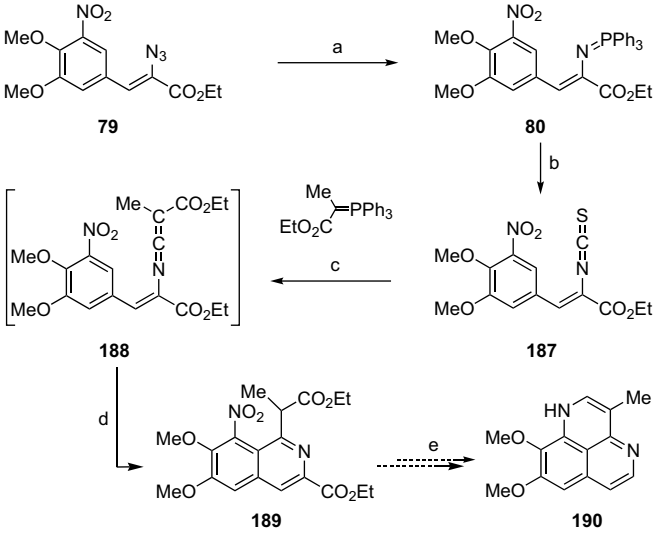

**Scheme 32.** Reagents and conditions: (a) NBS, NaOH, $CH_2Cl_2$, $H_2O$ (94%); (b) $CH_2(CO_2H)_2$, 120 °C (88%); (c) PPA, 150 °C, 75 min (79%); (d) $NaN_3$, $H_2SO_4$ (**184a**, 16%; **184b**, 76%); (e) $LiAlH_4$, THF, reflux, 30 min (90%); (f) 5% Pd/C, xylene, reflux, 2 h; (g) 1. $Al_2O_3$ chromatography; 2. THF, $HCl_{concd}$ (**179a**, 54%; **179b**, 31%).

The synthesis of **179b** (bis-demethoxyaaptamine) was aimed to furnish a model study for the preparation of other aaptaminoids and provide a deoxygenated analog of the natural products for biological studies (Scheme 32). For the synthesis, the commercially available 1,2,3,4-tetrahydroisoquinoline **180** was oxidized to 3,4-dihydroisoquinoline **181** with NBS/NaOH (94%),[134] which in turn was condensed with malonic acid to give 88% of the β-amino acid **182** after decarboxylation.[61]

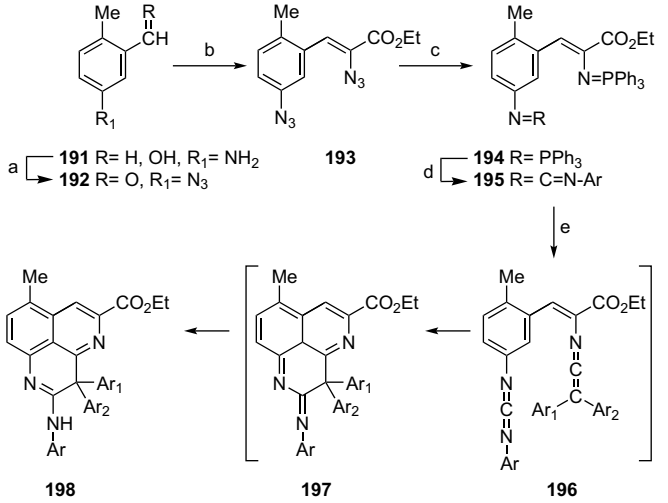
Next, cyclodehydration of **182** with excess PPA afforded the tricyclic amino ketone **183**[135] in good yield. As expected, the latter underwent a Schmidt reaction to give a separable 5:1 mixture of desired lactam **184b** and its isomer **184a** in combined 92% yield.[118a] The amino lactam **184b** was reduced cleanly with lithium aluminum hydride, and the resulting hexahydro product **185**, obtained in 90% yield, was dehydrogenated with Pd/C in refluxing xylenes to afford a mixture of the free base of bis-demethoxyaaptamine **179b** (31%) and the dihydrocompound **179a** (54%). Upon chromatographic separation, the free base **179a** was treated with HC1, yielding **179a**·HCl in 6.6% overall yield.




**Scheme 33.** Reagents and conditions: (a) 1. NaH, DMF; 2. MeI, DMF, rt, 1 h (**185a**, 19%; **185b**, 41%); (b) 1,2-$Cl_2$–$C_6H_4$, reflux, 2 h (73%); (c) see Scheme 20 (64% overall yield from **95**); (d) $K_2CO_3$, MeI, DMF, 100 °C, 2 h (68%); (e) 1. 10% Pd/C, $HCO_2NH_4$, MeOH, 40 min; 2. $(NH_4)_2SO_4$, $Et_3N$, HMDS, reflux, 20 h; 3. HCl, MeOH, rt, 20 h (73%).



**Scheme 34.** Reagents and conditions: (a) $PPh_3$, $Et_2O/CH_2Cl_2$, rt, 10 h (93%); (b) $CS_2$, PhH, reflux, 48 h; (c) $MeC(=PPh_3)CO_2Et$, PhMe, reflux, 1 h; (d) PhMe, 160 °C (sealed tube) 8 h (24%); (e) Cava's synthesis.



**Scheme 35.** Reagents and conditions: (a) 1. $NaNO_2$, $HCl/H_2O$, 0 °C; 2. $NaN_3$; 3. PCC, $CH_2Cl_2$, rt (83%); (b) $EtO_2CCH_2N_3$, NaOEt, EtOH, −16 °C (67%); (c) $PPh_3$, $Et_2O$, rt (91%); (d) ArNCO, PhMe, 160 °C, sealed tube; (e) $Ar_1Ar_2C{=}C{=}O$, PhMe, 160 °C, sealed tube (45–65%).

#### 4.5.2. Synthesis of methyl derivatives

Compound **175**, the unnatural *N*-methylaaptamine was independently synthesized by the groups of Hibino[99] and Sundberg,[105] while Pettit also disclosed its preparation from aaptamine en route toward isoaaptamine (Scheme 29).

Hibino's synthesis started with the methylation of quinoline-oxime **119** (Scheme 33), which gave a 1:2 mixture of methyloxime **185a** along with the quinoline derivative **185b** in combined 60% yield. Subsequent refluxing of a solution of **185a** in *o*-dichlorobenzene caused the electrocyclization of the 1-azahexatriene giving 73% of 1-methylaaptamine (**175**). In the approach of Walz and Sundberg, a minor modification to the synthesis of aaptamine,[105] consisting in the selective N-methylation of the quinolone ring of key intermediate **129** (prepared in 64% yield from aldehyde) gave 68% of derivative **186**, which resulted in 73% of **175**, after removal of the carboxybenzyl group and HMDS-assisted cyclization.

The unnatural methyl derivatives 1-methyl-8-demethylaaptamine (**160**) and 8-demethylaaptamine (**161**) were also prepared following the same general strategy, as shown in Scheme 25.

In addition, Molina et al.[79] synthesized the intermediate **189**, which may be used to obtain 3-methylaaptamine **190** (Scheme 34). To that end, the vinyl azide **79**, obtained by condensation of the known 5-nitroveratraldehyde (**95**)[91] with ethyl azidoacetate, was converted into iminophosphorane **80** by a Staudinger reaction[136] with triphenylphosphine in a $CH_2Cl_2/Et_2O$ solvent mixture. Exposure of the latter to carbon disulfide in refluxing benzene gave the corresponding thiocyanate **187**, which was transformed into intermediate **188** by an aza-Wittig reaction with carbethoxy-ethylidene triphenyl-phosphorane.[137] Finally, the required 1-substituted isoquinoline **189** was obtained through a thermally induced electrocyclic ring closure. According to the authors, this compound may be converted into 3-methylaaptamine (**190**) in a straightforward manner employing the Pelletier and Cava strategy.[57a]

In still another demonstration of the synthetic capabilities of the intramolecular version of the aza-Wittig-type reaction, the group of Molina[138] prepared a series of benzo[*de*][1,6]naphthyridines (Scheme 35). To that end, their common precursor bis-iminophosphorane **194** was prepared in three steps and 50% overall yield from alcohol **191** by diazotation and addition of sodium azide, followed by PCC oxidation to yield **192**, condensation with ethyl azidoacetate, leading to azido ester **193** and final Staudinger reaction with triphenylphosphine.

The aza-Wittig reaction between **194** and 1 equiv of phenylisocyanate led to iminophosphoranes **195**, bearing a carbodiimide moiety directly linked to an aromatic ring. Reaction of the latter with ketenes gave intermediates **196**, which were transformed into the tricyclic compounds **198** in reasonable yields, through the intermediacy of **197**.

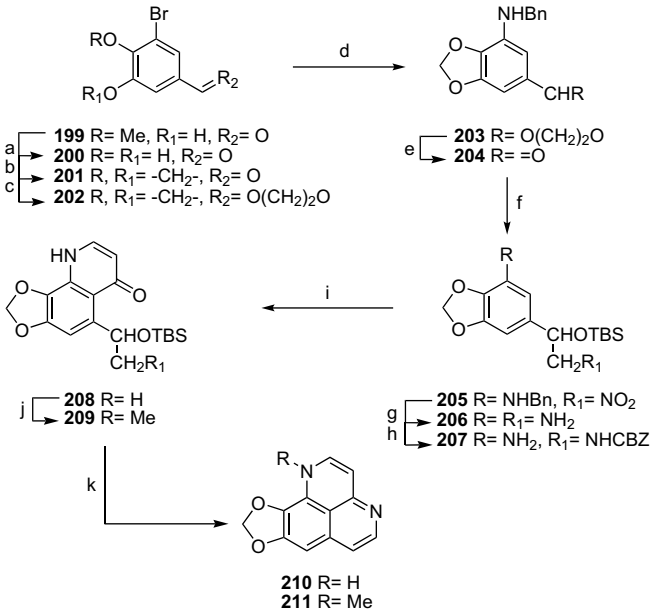
Presumably, the initial aza-Wittig reaction yielding **196** would be followed by an intramolecular hetero-Diels–Alder cycloaddition, with the aryl carbodiimide moiety acting as a 2-aza diene using one cumulative double bond and one carbon–carbon double bond of the aromatic ring, and the carbon–carbon bond of the styryl ketene imine group being the dienophile. The final tricyclic compound would result from re-aromatization of the cycloadduct **197** by way of a [1,5] proton shift.

#### 4.5.3. Synthesis of 1,3-dioxolane derivatives

Two 8,9-methylenedioxy derivatives of aaptamine (**210** and **211**) have also been targeted for synthesis (Scheme 36). The bromoaldehyde **199**[139] was transformed into the corresponding dioxolane **201** in 78% yield by way of catechol **200**, and the formyl moiety was acetalized with ethyleneglycol under tosic acid catalysis, furnishing acetal **202**. Buchwald's Pd-catalyzed amination conditions[140] gave benzylaminoacetal **203**, which was converted into aldehyde **204** after acid catalyzed removal of the dioxolane protecting group in excellent yields.

Nitroaldol addition of nitromethane under Amberlyst A-21 catalysis and the following O-silylation proceeded efficiently to give 86% of **205**. This was followed by simultaneous hydrogenolysis of the benzyl group and reduction of the nitro moiety under transfer hydrogenation conditions with 10% Pd/C and ammonium formate,[108] to access diamine **206**, which was selectively protected as the *N*-CBZ derivative **207** with CBZCl and potassium carbonate under DMAP promotion, in 90% yield.

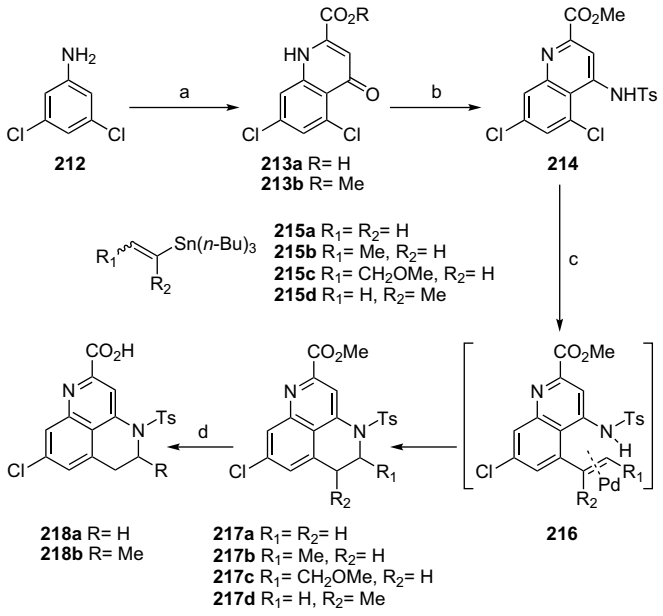
Finally, installation of the side chain and cyclization to quinolone **208** from aniline **207** were carried out employing the previously discussed strategy (Scheme 20) and the remainder of the synthesis, leading to the $N_1$-methyl (**211**) and the unsubstituted (**210**) methylenedioxy analogs of aaptamine followed a sequence similar to that conducting to **175** and aaptamine, respectively.



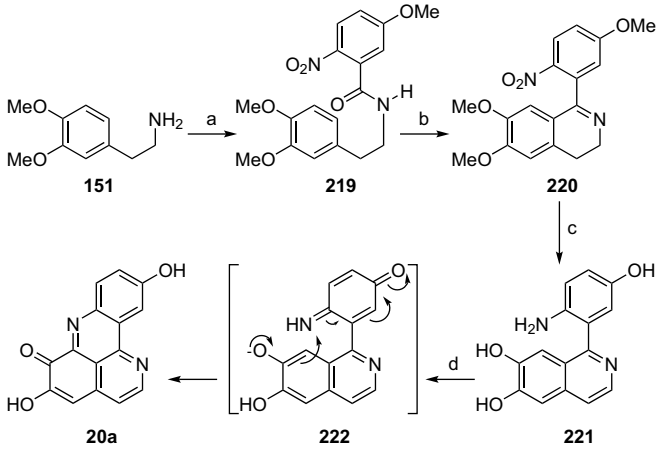

**Scheme 36.** Reagents and conditions: (a) $AlCl_3$, $C_5H_5N$, $CHCl_3$, 0 °C→reflux, 24 h (92%); (b) KF, $CH_2Br_2$, DMF, 140 °C, 4 h (85%); (c) $HOCH_2CH_2OH$, TsOH, PhH, reflux, 24 h (98%); (d) $BnNH_2$, $Pd_2(dba)_3$, *S*-BINAP, NaO*t*-Bu, PhMe, 90 °C, 3 h (96%); (e) PPTS, acetone/$H_2O$, reflux, 30 min (100%); (f) 1. $MeNO_2$, Amberlyst A-21; 2. TBDMSOTf, 2,6-lutidine, 0 °C→rt (86%); (g) 10% Pd/C, $HCO_2NH_4$, reflux, 2 h (98%); (h) CBZCl, $K_2CO_3$, DMAP, −78 °C to 30 °C, 8 h (91%); (i) $Ph_2O$, 240 °C, 20 min (67%); (j) $K_2CO_3$, MeI, DMF, 100 °C, 2 h, (96%); (k) 1. 10% Pd/C, $HCO_2NH_4$, MeOH, 40 min; 2. $(NH_4)_2SO_4$, $Et_3N$, HMDS, reflux, 20 h; 3. MeOH, HCl, rt, 20 h (**210**, 68%; **211**, 91%).

### 4.6. Synthesis tricyclic azakynurenic acids

Hume and Nagata prepared tricyclic azakynurenic acids **218a**,**b**, analogs of 5,7-dichlorokynurenic acid **213a** as a new class of *N*-methyl-D-aspartate (NMDA)-glycine site receptor antagonists, employing a Stille coupling reaction between sulfonamide **214**, readily available from 3,5-dichloroaniline (**212**) by way of quinolone ester derivative **213b**[141] and vinylstannanes (Scheme 37).[142]




**Scheme 37.** Reagents and conditions: (a) 1. dimethyl acetylene dicarboxylate, MeOH, reflux, 12 h (77%); 2. $Ph_2O$, 200 °C (90%); (b) TsNCO, MeCN, reflux, 80%; (c) **215**, 5% $Pd(PPh_3)_4$, NMP, 100–140 °C (**217a**, 63%; **217b**, 40%, **217c**, 18%), or $Pd_2(dba)_3$–$PPh_3$, NMP, 140 °C (**217c**, 13%), or $Pd_2(dba)_3$-P(2-furyl)$_3$, NMP, 140 °C (**217d**, 38%); (d) 1. $H_2SO_{4concd}$; 2. 1 N NaOH (>90%).




**Scheme 38.** Reagents and conditions: (a) 5-MeO–2-$NO_2$–$C_6H_3COCl$; (b) $POCl_3$, MeCN, 85–93%); (c) 1. $MnO_2$, PhH, reflux, 24 h (90–96%); 2. 48% HBr, reflux, 11 h (64%); 3. $H_2$, Pd/C (82–85%); (d) 5% $NaOH_{aq}$, $O_2$, rt, 12 h (67%).

Surprisingly, without the need of an oxidizing agent, the outcome of the Stille reaction was the cyclized products **217**, presumably formed through the intermediacy of palladium complexes **216**.[143] The reaction could be extended to other ester products **217**; however, yields were variable and careful optimization of the reaction conditions was required.

### 4.7. Total synthesis of necatorone

Steglich disclosed a total synthesis of necatorone (**20a**) a scarce and highly mutagenic pigment of *L. necator*, employing a Bischler–Napieralski based protocol (Scheme 38).[55] The synthesis involved condensation of homoveratrylamine (**151**) with 5-methoxy-2-nitro-benzoyl chloride to give the corresponding β-phenethylamide **219**, which upon treatment with $POCl_3$ in refluxing MeCN afforded 3,4-dihydroisoquinoline derivative **220** in good yield.

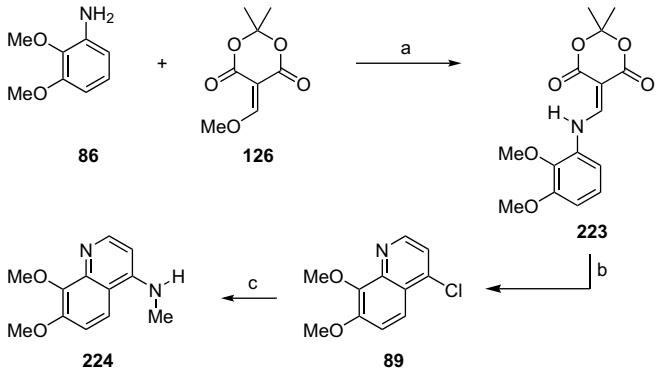
Dehydrogenation of **220** to the isoquinoline by refluxing with $MnO_2$ in benzene under azeotropic removal of water, followed by methyl ether cleavage with refluxing 48% HBr and catalytic hydrogenation with Pd/C provided the 4-aminophenol derivative **221**, which was oxidatively cyclized with 5% NaOH in the presence of air, presubmably through the intermediacy of quinone-imine **222**, furnishing the natural product in more than 25% overall yield. Further oxidation of **20a** with $H_2O_2$ and horseradish peroxidase gave the dimeric compound **20b**.

### 4.8. Synthesis of the pentacyclic ring system of lihouidine

Feldman and Coca[52] recently reported the synthesis of compound **17**, which embodies the pentacyclic core of lihouidine (**16**), through the assembly of two quinoline fragments and a novel nitration-promoted cyclization.

Amine **224**, one of the key quinoline fragments, was synthesized in three steps from aniline derivative **86**, by condensation with Meldrum's acid derivative **126**,[105] which afforded **223** (Scheme 39); in turn, upon refluxing in $Ph_2O$, this readily cyclized to known quinoline **88**, which was transformed (with $POCl_3$) to chloride **89** in 59% overall yield. Heating with methylamine in a sealed tube effected the nucleophilic aromatic substitution, furnishing 70% of **224**.

The second quinoline unit (**227**) was prepared in two steps by refluxing a mixture of isatin (**225**) and malonic acid in AcOH,[144] and converting the resulting 2-quinolone derivative **226** (90% yield) to the related 2-chloroquinoline by treatment with $POCl_3$, followed by an in situ esterification mediated by addition of MeOH (Scheme 40).




**Scheme 39.** Reagents and conditions: (a) $HC(OMe)_3$, 100 °C; (b) 1. $Ph_2O$, 259 °C; 2. $POCl_3$, 95 °C (59% overall); (c) 40% $MeNH_{2aq}$, 140 °C, sealed tube (70%).

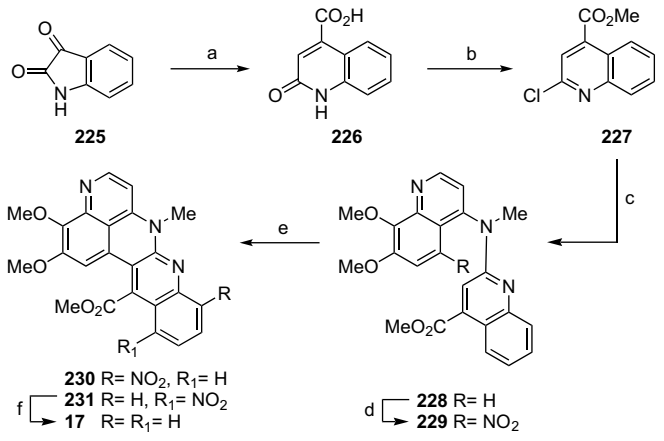
A Buchwald–Hartwig type coupling[145] between amine **224** and chloride **227** gave the bis-quinoline intermediate **228**. Serendipitously, the authors discovered that classical nitration conditions with the $HNO_3/H_2SO_4$ reagent, produced a small amount of pentacyclic nitro-derivatives **230** and **231**, in addition to uncyclized **229**.

Optimization of reagents and reaction conditions gave 52% of **231**, which was transformed into **17** in 42% yield by a three-step sequence consisting in an iron-mediated reduction of the nitro group to the amine, followed by diazotization and final $H_3PO_2$-mediated reductive cleavage of the diazonium moiety.

## 5. Biological activities of naturally occurring aaptamines, their analogs and derivatives

The potential of the aaptamines for drug development is evidenced by the results of the highly diverse group of molecular targets already evaluated. In addition, the simplicity of aaptamine's core structure and the potential for preparing orally bioavailable analogs provide unique opportunities for drug discovery.

However, compounds, which are active against a variety of targets are likely to encounter problems with indiscriminant toxicities and unwanted side effects. It would require some structural fine tuning in order to achieve improved specificity, should one of the aaptamine analogs or derivatives be further developed into a pharmaceutically active ingredient. Next, the different bioactivities found in aaptamine, its congeners and derivatives are briefly summarized, in an approximately increasing order to system complexity.




**Scheme 40.** Reagents and conditions: (a) $CH_2(CO_2H)_2$, AcOH, 105 °C (98%); (b) $POCl_3$, MeOH, 110 °C (32%); (c) **224**, $Pd_2(dba)_3$ (0.5 equiv), DCPB (0.1 equiv), $K_3PO_4$, PhMe, 110 °C (70%); (d) $Bu_4NNO_3$, TFAA, 0 °C→rt (71%); (e) $HNO_3$, $H_2SO_4$, 0 °C (**229**, 10%, **230**, 9%, **231**, 31%) or fuming $HNO_3$, 0 °C (**229**, 12%; **231**, 52%); (f) 1. $Fe^0$, 0.5 M HCl, EtOH, rt; 2. $NaNO_2$, 4 M HCl; 3. 50% $H_3PO_2$, 0 °C (42%).

### 5.1. Radical scavenging and antioxidant activities

Reactive oxygen species and oxidative stress play important roles in the etiology and progression of major human degenerative diseases.[146] Aaptamine (**1**), isoaaptamine (**2**), and demethylaaptamine (**3**) have been tested for their ability to scavenge the stable free 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and also hydroxyl radicals ($OH^{\bullet}$) forming in the Fenton reaction. In these assays, the tested compounds exhibited strong antioxidant activity (DPPH, $IC_{50}$=5.63, 2.50, and 1.25 μM, respectively, and $OH^{\bullet}$ scavenging, $IC_{50}$=5.1, 1.5, and 1.3 μM, respectively).

Takamatsu et al. observed that the antiradical activities of the aaptamines depend on number and position of the hydroxyl groups.[147] The tricycles were also submitted to the 2′,7′-dichlorodihydrofluorescein diacetate cellular-based assay, which detects only antioxidants that penetrate cellular membranes. However, **1**–**3** were relatively inactive ($IC_{50}$>55 μM).[147] This suggests that these compounds lack the capacity to quench 2′,7′-dichlorofluorescein fluorescence inside the cell, or perhaps they do not enter the cells due to poor cellular uptake or reduced solubility in the test medium.

### 5.2. Enzymatic inhibition activity

Aaptamine and its congeners were shown to act as inhibitors of different enzymes. The group of Fedoreev informed that isoaaptamine (**2**) caused 50% inhibition of 8-1,3-glucanase in fairly high concentration (50–60 μg/sample), the inhibiting effect being three times greater than that of aaptamine itself.[148] The same group disclosed that aaptamine is a monoamine oxidase (MAO) inhibitor, able to block the deamination of serotonin ($IC_{50}=10^{-5}$ M) not only in the human placenta, where MAO-A predominates, but also in the rat liver, where the ratio between MAO-A and MAO-B is approximately 1:1. In addition, it was observed that the deamination of benzylamine, the specific substrate of MAO-B, either was not completely blocked or it was inhibited to a minor degree in comparison with the oxidative deamination of serotonin, the specific substrate of MAO-A.[149]

Aaptamine was also found to be an inhibitor of glutamine: fructose-6-phosphate amidotransferase (GFAT) during a high throughput screening program searching for enzymatic inhibitors in natural products' extracts ($IC_{50}$=120 μM).[36] GFAT is an attractive target for the discovery of drugs, which may be used for the treatment of type II diabetes. In addition, Badet-Denisot et al. recently reported that aaptamine is a weak inhibitor of the bacterial enzyme glucosamine-6-phosphate synthase (GLMS).[36,150] This enzyme is a pharmacologically interesting target, because it catalyzes the first committed step of hexosamine metabolism, yielding an essential building block for bacterial and fungal cell walls.

On the other hand, the serine/threonine protein kinase isoenzyme family, protein kinase C (PKC), is a group of closely-related enzymes, which apparently intervene in a range of responses in different cell types, playing a significant role in facilitating effects of hormones, neurotransmitters, growth factors, antigens, and inflammatory mediators. Their involvement in cellular proliferation and differentiation has made inhibitors of these isoenzymes of great interest as potential treatments for cancers, cardiovascular, renal or central nervous system disorders, inflammation, immunosuppression, and septic shock.[151]

Following a previous patent report on the PKC inhibition activity of isoaaptamine,[152] Pettit et al. tested isoaaptamine (**2**), 9-demethylaaptamine (**3**), 8,9-bisdemethylaaptamine (**5**), 4-methylaaptamine (**10**), the monobenzyl compounds **166**–**168**, as well as the

quaternarized derivatives **164**, **165**, **169**, **170**, and **171**, as ligands for PKC, concluding that they showed no appreciable affinity for the enzyme.

However, when the ability of these derivatives to inhibit PKC catalytic activity was evaluated, marked inhibition was observed for compounds **164** and **165** (71.1 and 84.6%, respectively), **169** and **170** exhibited less inhibition, while the remaining compounds were essentially inactive.[125] On the other hand, the group of Longley analyzed the ability of a series of compounds to effect signal transduction in a newly developed cell adhesion assay using the EL-4 cell line; they observed that demethyl(oxy)aaptamine (**6**) was capable to affect EL-4.IL-2 cell adhesion,[153a] this being correlated to protein kinase C (PKC) agonism and antagonism.[153b]

Recently, several aaptaminoids have also been reported as interesting sortase A inhibitors. Sortase A has been identified in *Staphylococcus aureus* and shown to be related to cell wall anchoring of protein A and to the virulence of this bacterium. Because many of the known surface proteins of Gram-positive bacteria are believed to be exported and anchored via the sortase pathway, inhibitors of this enzyme may be promising candidates for the treatment and/or prevention of Gram-positive bacterial infections.[154]

Sortase A inhibitors should act as anti-infective agents and disrupt the pathogenesis of bacterial infections without affecting microbial viability. The group of Oh[48] informed the Sortase A bioassay guided isolation of **1**–**3** and **6** from *A. aaptos*; these compounds inhibited the enzyme in *S. aureus* Newman strains with $IC_{50}$ values of 23.5, 3.7, 17.2, and 20.1 μg/mL, respectively. In addition, treatment of the Newman strain with isoaaptamine (**2**) reduced the capacity of the bacterium to adhere to fibronectin-coated surfaces in a dose-dependent manner. The onset and magnitude of the inhibition of fibronectin-binding in *S. aureus* Newman treated with **2** was comparable to the behavior of an untreated mutant, devoid of sortase A and 100 times less virulent.

### 5.3. Antiviral activity

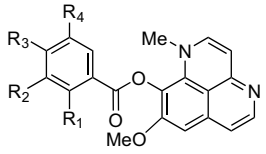
Interestingly, demethyl(oxy)aaptamine (**6**) and 4-methylaaptamine (**10**) were found to inhibit replication of HSV-1 in Vero cells ($EC_{50}$=2.4 μM),[37,155] proving to be more active than acyclovir ($EC_{50}$=8.6 μM), and more efficient than other previously tested alkaloids.[156] The tricycles were non-cytotoxic below 20 μM ($CC_{50}$=72 μM).

Apparently, the aaptamines target immediate-early protein ICP 27, which regulates splicing, termination, and nuclear export events of viral transcripts, resulting in impairment of all other steps of HSV-1 replication; this also affects additional targets such as viral penetration, differing in its mechanism of action from acyclovir.

Gul et al. tested aaptamine derivatives against HIV-1 in human peripheral blood mononuclear (PBM) cells,[132] finding that demethyl(oxy)aaptamine (**6**) exhibited good performance ($EC_{50}$= 0.34 μM), meaning to be only 100 times less potent than the 3′-azidothimine (AZT) control, while aaptamine (**1**) remained poorly active ($EC_{50}$=1.3 μM) and isoaaptamine (**2**) showed moderate activity ($EC_{50}$=0.6 μM). These authors also modified the 9-position of isoaaptamine, synthesizing various esters (Table 2). The position of fluorine atoms and the alkyl chain length of *para*-substituted benzoates were the main determinants of variations in their activity.

Simultaneously, cytotoxicity for PBM, T-lymphoblastoid (CEM), and African green monkey kidney (Vero) cell lines were measured to determine the therapeutic selectivity of active derivatives. However, cytotoxicity for the majority of the compounds was high in comparison to the control. Thus, although the potency of **6** looked promising, this compound ranked among the most cytotoxic in this study ($IC_{50}$=1.2 μM).[27,157]

**Table 2**
Anti-HIV-1 activity of **1**, isoaaptamine and its esters **232a**–**m**



**232**

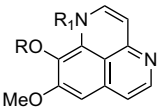
| Compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $EC_{50}$ (μM) | $EC_{90}$ (μM) |
|---|---|---|---|---|---|---|
| **1** | | | | | 0.6 | |
| **2** | | | | | 1.3 | |
| **232a** | H | H | Me | H | 9.2 | 29.1 |
| **232b** | H | H | $CH_2Me$ | H | 0.5 | 3.0 |
| **232c** | H | H | $(CH_2)_3Me$ | H | 3.8 | 7.1 |
| **232d** | H | H | $(CH_2)_4Me$ | H | 18.8 | 66.4 |
| **232e** | F | H | H | F | 2.1 | 10.5 |
| **232f** | H | H | $OCF_3$ | H | 2.3 | 16.3 |
| **232g** | H | H | F | H | 10.9 | 33.5 |
| **232h** | H | F | F | H | 16.6 | 54.3 |
| **2321** | F | F | H | H | 37.7 | 70.6 |
| **232m** | H | OMe | H | H | 33.7 | 60.1 |

### 5.4. Antimicrobial, antifungal and antiparasitic activities

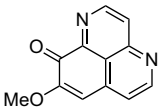
Crude extracts of different *Aaptos* sponge specimens have shown to possess antimicrobial activity against different bacterial strains, while some extracts also displayed weak antimicrobacterial properties.[158] Nakamura found that demethyl(oxy)aaptamine (**6**) exhibited the most potent antimicrobial activity against Gram-positive and Gram-negative bacteria such as *S. aureus* (MIC=3.13 μg/mL), *Bacillus subtilis* (MIC=6.25 μg/mL) and *Proteus vulgaris* (MIC 12.5 μg/mL). Antimicrobial activities of 9-demethylaaptamine (**3**) were approximately half of these.[27]

The group of Calcul evaluated the antimicrobial activity of aaptamine (**1**), isoaaptamine (**2**), demethyl(oxy)aaptamine (**6**), its dimethylketal (**11**), and the tetracyclic aaptaminoids **12**–**15** toward Gram-positive (*S. aureus*), Gram (−) (*Escherichia coli, Vibrio anguillarum*) bacterial strains, and the yeast *Candida tropicalis* (Table 3).[38] Compounds **1** and **2** showed moderate antibacterial

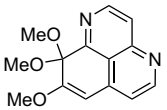
**Table 3**
Effect of compounds **1**, **2**, **6**, and **11**–**15** on the growth of selected microbial strains and the yeast *C. tropicalis*



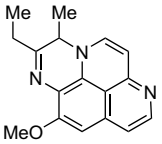
**1** R= Me, $R_1$= H
**2** R= H, $R_1$= Me



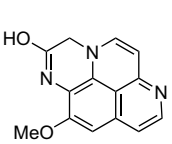
**6**



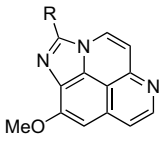
**11**



**12**



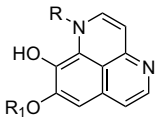
**13**



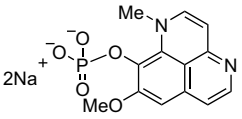
**14** R= $CH(CH_3)_2$
**15** R= H

| Compound | MIC (μg/mL) | | | |
|---|---|---|---|---|
| | *S. aureus* | *E. coli* | *V. anguillarum* | *C. tropicalis* |
| **1** | 25 | >100 | 12 | 25 |
| **2** | 6 | >100 | 12 | 12 |
| **6** | 25 | 100 | 100 | >100 |
| **11** | >100 | >100 | >100 | >100 |
| **12** | >100 | >100 | >100 | >100 |
| **13** | >100 | >100 | >100 | >100 |
| **14** | >100 | >100 | >100 | >100 |
| **15** | >100 | >100 | 100 | >100 |

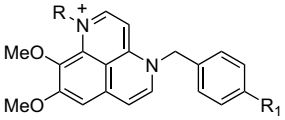
**Table 4**
Effect of different aaptaminoids on the growth of selected microbial and yeast strains



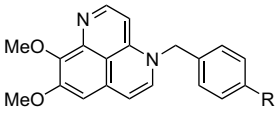
**2** R= $R_1$= Me
**3** R= H, $R_1$= Me
**5** R= $R_1$= H



**163**



**164** R= Bn, $R_1$= H
**165** R= 4-MeO-$C_6H_4CH_2$, $R_1$= OMe
**174** R= Me, $R_1$= OMe



**166** R= H
**167** R= OMe

| Microorganism | Minimum inhibitory concentration (MIC) range (μg/mL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **2** | **3** | **5** | **163** | **164** | **165** | **166** | **167** | **174** |
| *C. neoformans* | 64 | 32 | 32 | NA | NA | 32–64 | NA | NA | NA |
| *C. albicans* | NA | 64 | 64 | NA | NA | NA | NA | NA | NA |
| *S. aureus* | 16–32 | 16 | 4-8 | 64 | NA | 4 | NA | NA | NA |
| *S. pneumoniae* | 8–32 | 2-4 | 8 | 16–32 | 32 | 16–32 | 64 | 64 | NA |
| *E. fecalis* | 32–64 | 8–32 | 8–16 | NA | 32 | 16–32 | NA | NA | NA |
| *M. luteus* | 32–64 | 8 | 4 | NA | 0.5–2 | <0.5 | NA | 64 | 4–8 |
| *E. coli* | NA | 32–64 | 16–64 | NA | 8 | NA | NA | NA | NA |
| *E. cloacae* | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| *S. maltophilia* | NA | NA | 32 | | NA | NA | NA | NA | NA |
| *N. gonorrheae* | <0.5 | <0.5 | <0.5 | <0.5 | 0.25 | 4 | 64 | 64 | 64 |

NA=not active.

activity, followed by **6** and **15**. Notably, they were active against the ichthyopathogenic strain *V. anguillarum*, while aaptaminoids **11**–**14** were inactive against the tested bacteria and yeast and only compounds **1** and **2** exhibited antifungal activity toward *C. tropicalis*.

Pettit et al. reported that isoaaptamine (**2**), 9-demethylaaptamine (**3**), and 8,9-bisdemethylaaptamine (**5**) exhibited antimicrobial activity against clinically relevant microorganisms (Table 4). Hystatin 1 (**163**), a prodrug of isoaaptamine (**2**) retained part of the antimicrobial activity of the parent compound.[126] On the other hand, *N*-benzyl derivatives **166** and **167** were marginally active against *Streptococcus pneumoniae* and *Neisseria gonorrheae* ($IC_{50}$=64 μg/mL), whilst **164** and **165** inhibited the clinically important microorganisms *Micrococcus luteus* ($IC_{50}$=0.5–2 μg/mL) and *N. gonorrheae* ($IC_{50}$=0.25–4 μg/mL).[125] Pettit has also found antifungal activity against *Cryptococcus neoformans*[125] in compound **165**, whilst 9-demethylaaptamine (**3**) and 8,9-bisdemethylaaptamine (**5**) were active against *Candida albicans* and *C. neoformans* ($IC_{50}$=32–64 μg/mL).[126] Interestingly, necatorone (**20a**) also showed moderate antibiotic activity against *B. subtilis*, *B. brevis*, and *Acetobacter calcoaceticus*.[55c]

Bowling et al. studied the activity of different semisynthetic aaptaminoids against microbial and AIDS-related opportunistic infection agents. In their assay, aaptamine, isoaaptamine, and 4-methylaaptamine (**10**) exhibited $IC_{50}$ values above 35.4 μM, being considered inactive.[131]

Their data (Table 5) showed that several *N*-alkyl analogs were potent against bacteria and moderately active as antifungal agents. Interestingly, derivatives **233** with side chains having less than five carbon atoms were inactive, but compounds like **233c** and **233e** were active against the methicillin-resistant strain of *S. aureus* (MRSa).[159]

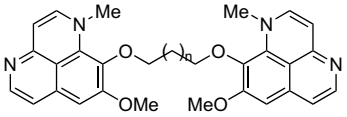
Based on SAR considerations, the activity of these compounds was correlated to their log *D* values, concluding that among these analogs, chain length should produce a log *D* between 3.75 and 7.09 for them to be effective against MRSa.

However, when these derivatives were tested against *Mycobacterium tuberculosis*, they were moderately active at best. On the other hand, isoaaptamine (**2**) was inactive while isoaaptamine ester **232m** exhibited the best performance ($IC_{50}$=41.0 μg/mL).[132] Pettit et al. also evaluated mono- and bis-benzyl derivatives **166**–**168** and **164**, **165**, **169**, and **170** against *M. tuberculosis*. At 6.25 μg/mL, compounds **164** and **165** were active, exhibiting 98% and 97% inhibition, respectively.[125]

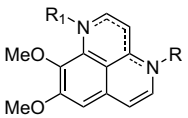
Bowling et al. also found interesting activity among several aaptamine derivatives, against chloroquine sensitive (D6) and resistant (W2) *Plasmodium falciparum* strains (Table 6). $N_1$,$N_4$-Bisalkyl aaptamine derivatives, having more than five carbon units in their alkyl chains, were specially active; however, a distinct improvement in the activity was seen with compound **234**.

Demethylation of the C-9 methoxy group of **233d** produced over a 10-fold increase in activity against the *P. falciparum* resistant strain, supporting previous observations, indicating that protection of the C-9 hydroxyl generally decreases potency.[131] When tested against *Leishmania donovani*, isoaaptamine (**2**) was more active ($IC_{50}$=0.68 μg/mL) than pentamidine and amphotericin B reference drugs. Modification of **2** decreased activity, except for esters **232a** and **232b**, which outperformed the parent isoaaptamine ($IC_{50}$=0.1 and 0.4 μg/mL, respectively).[132]

**Table 5**
Efect of bis-aaptaminoids **178a**,**b** and isoaaptamine derivatives **220a**–**e** against microbial and AIDS related opportunistic infections



**178a** n= 4; **178b** n= 7

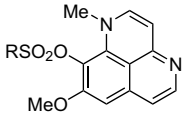


**233a** R= H, $R_1$= Heptyl
**233b** R= Heptyl, $R_1$= H
**233c** R= Hexadecyl, $R_1$= H
**233d** R= $R_1$= Dodecyl
**233e** R= H, $R_1$= Dodecyl

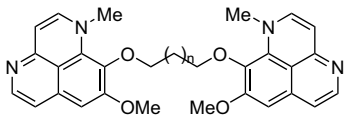
| Microorganism | $IC_{50}$ (μM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **178a** | **178b** | **213a** | **233b** | **233c** | **233d** | **233e** |
| *C. albicans* | 18.6 | 5.6 | 30.6 | NA | 6.6 | 17.7 | NA |
| *C. neoformans* | NA | NA | 13.8 | 45.8 | 3.3 | NA | 1.8 |
| *C. glabrata* | NA | 6.9 | NR | NR | 3.3 | 15 | NR |
| *C. krusei* | 27.8 | 5.6 | NR | NR | 7.7 | 11.5 | NR |
| *S. aureus* | NR | NR | 13.8 | 30.6 | NR | NR | 2.0 |
| MRSa[a] | 18.6 | 24.1 | 15.3 | 30.6 | 3.3 | 26.5 | 1.8 |
| *M. intracellulare* | 18.6 | 4.0 | 6.1 | 30.6 | 0.6 | NA | 0.8 |
| *A. fumigatus* | NA | 24.1 | 61.1 | NA | 9.9 | NA | 50.3 |

[a] Methicillin-resistant *S. aureus*.

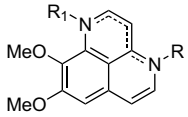
**Table 6**
In vitro activity of aaptamine and aaptaminoid derivatives against chloroquine sensitive (D6) and resistant (W2) malarial strains of *P. falciparum*



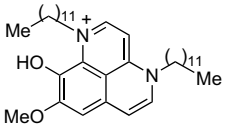
**177**



**178a** n= 4; **178b** n= 7



**233**



**234**

| Compound no. | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|
| | R | $R_1$ | Pf-D6 | Pf-W2 |
| **1** | | | NA | NA |
| **2** | | | 5.5 | NA |
| **3** | | | 8.2 | 11.9 |
| **5** | | | NA | NA |
| **177a** | Me | | 8.2 | NA |
| **177b** | Propyl | | 5.4 | 5.4 |
| **177c** | Octyl | | 6.2 | 6.9 |
| **177d** | Bn | | NA | NA |
| **178a** | | | 0.4 | 0.5 |
| **178b** | | | 0.4 | 0.5 |
| **233c** | Hexadecyl | H | NA | NA |
| **233d** | Dodecyl | Dodecyl | 2.5 | 3.9 |
| **233e** | H | Dodecyl | 1.2 | 2.0 |
| **233f** | H | Et | 3.1 | 8.6 |
| **233g** | Et | H | NA | NA |
| **233h** | H | Propyl | 7.4 | 7.4 |
| **233i** | Propyl | H | NA | NA |
| **233j** | Hexyl | H | 2.3 | 2.3 |
| **233k** | H | Hexyl | 0.6 | 0.9 |
| **233l** | Nonyl | H | 1.9 | 2.7 |
| **234** | | | 0.6 | 0.2 |

NA=not active.

On the other hand, biofilms are at the root of many infections largely because of their higher antibiotic resistance. Antibiotics targeting biofilm phenotypes are desperately needed, but there is still no standard method to assess biofilm drug susceptibility. The group of Pettit employed the ability of aaptamine derivative Hystatin 3 (**165**) to eradicate biofilms to devise such a method, correlating Alamar blue reduction with 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT) reduction and viable microorganism counts (CFU/mL) for different strains of *Staphylococcus epidermidis*, *a* major Gram-positive biofilm former. The dye both fluoresces and changes color in response to chemical reduction, and the extent of the conversion is a reflection of cell viability. For the tested strains, Alamar blue results correlated well with XTT and with CFU/mL results and **165** exhibited an MBIC of 64 μg/mL.[160]

### 5.5. Cytotoxic activity

Fedoreev et al.[28] found that the cytotoxic activity of alcoholic extracts from the Suberitidae sponges was due to the presence of aaptamine derivatives. However, the viability of Ehrich's tumor cells incubated with aaptamine at a dose of 50 μg/mL was not affected by the compound itself. *O*-Acetyl isoaaptamine (**176a**), at a dose of 25 μg/mL inhibited tumor cell growth in mice by 58%. However, the greatest tumor growth inhibition (95%) was observed when the cells were treated with demethylaaptamine (**3**) and isoaaptamine (**2**). The latter had the greatest effect on tumor cell viability; this was related by the authors to its improved ability to disturb cytoplasm membrane permeability. Aaptamine and *O*-acetyl isoaaptamine (**176a**) at a concentration of 32 μg/mL did not induce potassium ion loss from tumor cells, whereas $K^+$ loss was induced by preparations of **3** and **2** at concentrations of 16 and 8 μg/mL, respectively.

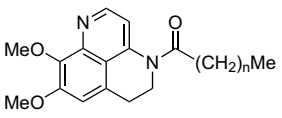
Nakamura studied the activity of aaptamine (**1**), 9-demethylaaptamine (**3**), and demethyl(oxy)aaptamine (**6**) against HeLa cells, and found that the latter was the most cytotoxic ($EC_{50}$=0.87 μg/mL); the activity of **3** was approximately half of that.[27] Lihouidine (**16**) also demonstrated to be moderately active against P388 cells ($IC_{50}$=3 μM).[51]

The group of Hamann explained the cytotoxicity of aaptamine by its ability to intercalate DNA, as assessed by a titration experiment monitored by UV–vis absorbance; the $K_{obs}$ was $4.0\times10^3$ $M^{-1}$, similar to that of the known DNA intercalator *N*-[2-(diethylamino)ethyl]-9-amino acridine-4-carboxamide.[131] The authors suggested that aaptamine utilizes DNA intercalation in its mechanism of action as antiviral and cytotoxic. The natural product binds with DNA in the same fashion as amsacrine. Interestingly, the group of Arif informed that at 10 μM, aaptamine induced benzo[*a*]pyrene/DNA adduction by two-fold in MCF-7 cells.[161] At this concentration, other compounds such as manzamine A, sarcophine, verongiaquinol, curcuphenol, and curcudiol were ineffective.

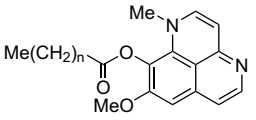
Calcul et al.[38] evaluated the antitumor activity of aaptamine (**1**), isoaaptamine (**2**), demethyl(oxy)aaptamine (**6**), and its dimethylketal (**11**) against KB cells, observing significant cytotoxic activity for **1**, **2**, and **6** ($ID_{50}$=3.7, 0.5, 1.8, and 3.5 μg/mL, respectively), in agreement with previous reports.[130]

Shen et al. prepared a series of acyl derivatives (**176**) of isoaaptamine (**2**) and 2,3-dihydroaaptamine (**21**) and tested their cytotoxic activity against four tumor cell lines (Table 7).[130] Compounds **1**, **2**, and **6** demonstrated to be significantly active against KB16, A549, HT-29, and P388, while, in general, the acyl analogs were less potent. This cytotoxicity study showed that the introduction of an acyl side chain at C-9 or *N*-4 decreased the activity; however the cytotoxicity of these compounds was somehow related to the number of carbon atoms in these side chains. In the

**Table 7**
Cytotoxic activity of aaptaminoids against four tumor cell lines



**22**



**176**

| Compound | *n*[a] | $IC_{50}$ (μg/mL) | | | |
|---|---|---|---|---|---|
| | | P388 | KB16 | A549 | HT-29 |
| **1** | | 0.6 | 3.9 | 2.8 | 6.9 |
| **2** | | 0.04 | 0.4 | 0.3 | 0.4 |
| **6** | | 0.01 | 0.1 | 0.3 | — |
| **19** | | — | — | >50 | >50 |
| **21** | | 1.8 | 22 | 23 | 47 |
| **22a** | 0 | 2.6 | 32 | 20 | 17 |
| **22b** | 4 | 3.7 | >50 | >50 | >50 |
| **22c** | 10 | 0.1 | 7.8 | 5.6 | 9.6 |
| **22d** | 16 | 2.7 | 20 | >50 | >50 |
| **176a** | 0 | 4.2 | >50 | >50 | >50 |
| **176b** | 4 | 0.7 | 33 | 13 | 12 |
| **176c** | 7 | 0.04 | 33 | 12 | 3.0 |
| **176d** | 8 | 0.03 | 20 | 6.1 | 2.5 |
| **176e** | 10 | 0.03 | 20 | 3.7 | 2.5 |
| **176f** | 12 | 0.3 | 3.3 | 5.6 | 2.7 |
| **176g** | 16 | 1.1 | >50 | >50 | 21 |

[a] *n*=Number of methylene groups in the acyl side chain.

P388 assay the activity of compounds **176c**–**176e** containing side chains of 7, 8, and 10 methylene groups, respectively, neared that of aaptamine. Other analogs, bearing shorter or longer side chains were less potent.

These results also pointed out to the importance of the presence of a free hydroxyl group at C-9. Its oxidation to give a carbonyl function such as compound **6** resulted in an increase of activity. Aaptamine, which contains a methoxyl group was also less active than isoaaptamine (**2**). 2,3-Dihydroaaptamine (**21**), a product obtained from hydrogenation of **1** also was showed to be less active than the latter, indicating that aromaticity in ring B is still important for activity. On the other hand, the decrease in activity of acylated products against human tumor cells was explained by their low water solubility and low bioavailability.

The research team of Longley evaluated 24 metabolites derived from marine sponges for their cytotoxicities against human non-small cell lung carcinoma A549, human colon adenocarcinoma HT-29, and murine leukemia cell line P388.[153a] In this study, 9-demethylaaptamine (**3**) expressed selective cytotoxicity against P388 relative to A549, while demethyl(oxy)aaptamine (**6**) proved to be non-selective.

The group of Pettit found that isoaaptamine (**2**) showed significant cytotoxicity against the murine P388 lymphocytic leukemia cell line ($ED_{50}$=0.28 μg/mL) and against a panel of six human cancer cell lines.[126] In a more extensive study, semisynthetic derivatives were prepared and tested (Table 8). It was observed that O-demethylation led to an increase in inhibitory activity, while methylation of aaptamine at N-4 gave the inactive derivative **10**, while mono-benzylated (derivatives **166**–**168**) exhibited increased cytotoxic activity.

The $N_1$,$N_4$-bismethyl derivative **171** was more active ($ED_{50}$=3.9 μg/mL against murine P388) than its $N_4$-methyl congener **10**, whilst the analog quaternary benzo[*de*][1,6]-naphthyridinium salts **164** (Hystatin-2) and **165** exhibited significant inhibitory activity against the murine P388 lymphocytic leukemia and human cancer cell lines, and salts **169** and **170** were inactive. It was also observed that unexpectedly, increasing the number of methoxy groups in both series **166**–**168** and **164**, **165**, and **169** led to a decrease in the cancer cell growth inhibitory activity.[125]

Gul et al. prepared a series of 21 isoaaptamine esters, mainly sustituted benzoate derivatives **232**, which were tested against a panel of 14 cancer cell lines. These authors found that the compounds displaying the broadest activity contained *para*-substituted phenyl rings. Of special interest were **232c** and **232d**, which inhibited growth of cell line K-562 with $GI_{50}$ values of 1.66 and 0.05 μM, respectively.[132]

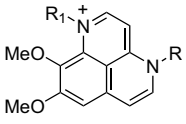
The prodrug Hystatin 1 (**163**) a stable derivative prepared to overcome slow degradation of isoaaptamine (**2**), retained some of the P388 cytotoxic activity of the parent compound, as shown in Table 9.[42] These aaptamine derivatives were also examined for potential effects on tubulin assembly.[126] Except for isoaaptamine (**2**) and 9-demethylaaptamine (**3**), which weakly inhibited the extent of tubulin assembly ($IC_{50}$=31 μM and 37 μM, respectively), no significant activity was observed among the aaptaminoids at the highest concentration evaluated (40 μM). Therefore, it may be concluded that cytotoxicity of these compounds is not a result from interaction with tubulin.

In a flow cytometry cell cycle analysis of THP-1 human monocytic leukemia cells, stained with propidium iodide and pretreated with 0.25 μg/mL Hystatin 2 (**164**), the accumulation of cells in the G1 phase was observed, whereas a cDNA microarray assay with THP-1 cells treated with **164** demonstrated significant down-regulation of several genes whose products are involved in DNA synthesis, suggesting that **164** may block the S-phase of the cell cycle.

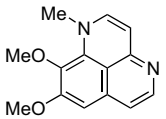
Shaari et al. screened a series of marine sponges for cytotoxic activity against a panel consisting of HL-60, CEM-SS, MCF-7, HeLa, HT-29, and L929 cell lines. They found the crude methanolic extract of a Malaysian *A. aaptos* to be active against all the cell lines ($CD_{50}$=3.2–24.1 μg/mL). Bioassay guided fractionation of the extract allowed the isolation of **1**, **8**, and **9**, which were tested against the CEM-SS (T-lymphoblastic leukemia) cell line, exhibiting $CD_{50}$ values of 15.0, 5.3, and 6.7 μg/mL, respectively.

Aaptamine, at concentrations in the range of 20–50 μM, has been also found to increase the expression of the cyclin dependent kinase inhibitor p21,[45] a protein known to act as a negative regulator of the cell cycle progression, hindering abnormal cell proliferation. Under normal circumstances, activation of p21 is dependent from p53, the mutation of which is one of the major events in carcinogenesis.[162] The activity of aaptamine on p21 was found to be p53-independent, arresting the cell cycle at the G2/M phase; therefore, from the 'gene-regulating chemotherapy or prevention' approach, it might contribute to cancer prevention or

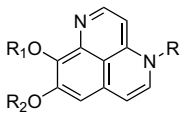
**Table 8**
Cytotoxic activity of natural compounds **2**, **3**, **5** and **10** and semisynthetic aaptaminoids **164**–**175** against a panel of tumor cell lines



**164** R= $R_1$= Bn
**165** R= $R_1$= PMB
**169** R= $R_1$= 3,4,5-$(MeO)_3$Bn
**170** R= 3,4,5-$(MeO)_3$Bn, $R_1$= 3-OH,4-MeOBn
**171** R= $R_1$= Me
**174** R= PMB, $R_1$= Me



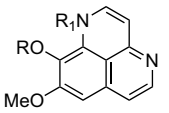
**175**



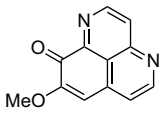
**166** R= Bn, $R_1$= $R_2$= Me
**167** R= PMB, $R_1$= $R_2$= Me
**168** R= 3,4,5-$(MeO)_3$Bn, $R_1$= $R_2$= Me
**172** R= Me, $R_1$= $R_2$= H
**173** R= $R_2$= Me, $R_1$= H

| Compound | $EC_{50}$ (μg/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | P388 | BXPC-3 | MCF-7 | SF-268 | NCI-H460 | KM-20L2 | DU-145 |
| **2** | 1.6 | 4.1 | 2.2 | 2.2 | 2.9 | >10 | 3.3 |
| **3** | 0.22 | 1.6 | 0.7 | 1.4 | 2.0 | 1.7 | 1.8 |
| **5** | 0.12 | 0.33 | 0.31 | 0.8 | 0.52 | 0.3 | 1.1 |
| **10** | >10 | >10 | >10 | >10 | >10 | >10 | >10 |
| **164** | 0.23 | 0.06 | 0.3 | 0.06 | 0.26 | 0.06 | 0.04 |
| **165** | 1.3 | 0.1 | 0.4 | 0.27 | 0.33 | 0.13 | 0.1 |
| **166** | 1.64 | 4.3 | >10 | >10 | 4.9 | 2.2 | 4.4 |
| **167** | 2.2 | 4.2 | 8.0 | 8.2 | 5.1 | 3.9 | 3.1 |
| **168** | 4.64 | >10 | >10 | >10 | >10 | 3.4 | >10 |
| **169** | >10 | >10 | >10 | >10 | >10 | >10 | >10 |
| **170** | >10 | >10 | >10 | >10 | >10 | >10 | >10 |
| **171** | 3.9 | 7.1 | 4.9 | 0.7 | >10 | 6.1 | 3.5 |
| **172** | 2.2 | >10 | 4.1 | 5.6 | >10 | >10 | >10 |
| **173** | >10 | >10 | >10 | >10 | >10 | >10 | >10 |
| **174** | 0.2 | 0.7 | 2.2 | 0.3 | 1.9 | 0.9 | 0.5 |
| **175** | 3.1 | 7.0 | 8.0 | 5.9 | 4.6 | 5.4 | 7.5 |

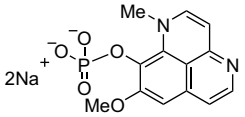
**Table 9**
Cytotoxic activity of aaptaminoids against a panel of seven tumor cell lines



**1** R= Me, $R_1$= H
**2** R= H, $R_1$= Me



**6**



**163, hystatin 1**

| Compound | $EC_{50}$ (μg/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | P388 | Ov-CAR-3 | SF-295 | A498 | NCI-H460 | KM-20L2 | SK-MEL-5 |
| **1** | 3.60 | 4.90 | 4.10 | 3.20 | 3.20 | 3.60 | 4.30 |
| **2** | 0.28 | 1.20 | 2.60 | 2.20 | 2.40 | 2.30 | 1.60 |
| **6** | 0.31 | 0.39 | 2.80 | 4.20 | 2.30 | 2.10 | 1.00 |
| **163** | 3.0 | — | — | — | >10 | >10 | — |

treatment.[163] However, in an independent study, the group of Arif recently demonstrated that aaptamine (10 μM) is also capable to enhance by 40% the expression of p53.[164]

### 5.6. α-Adrenergic antagonistic activity

Kobayashi et al. first suggested that aaptamine is a competitive antagonist of α-adrenoceptors in vascular smooth muscles, after treating rabbit isolated aorta and renal artery with the drug at a concentration of $3\times10^{-5}$ M. This produced a parallel, rightward shift of the dose–response curve for noradrenaline, whereas those for histamine or KCl remained unaffected. 9-Demethylaaptamine (**3**), demethyl(oxy)aaptamine (**6**), 2,3-dihydroaaptamine (**21**), and 2,3-dihydro-9-demethylaaptamine (**25**) had no effect on the dose–response curve for noradrenaline even at $10^{-4}$ M doses.[165a] Due to these antagonistic effects on α-adrenergic receptors, a cardiac activity has been ascribed to aaptamine. Sympatholytic and hypotensive activities have also been attributed to the natural product.[165b]

### 5.7. NMDA receptor inhibitory activity

The NMDA receptor plays a major role in excitatory signal transmission. Overexcitation of the NMDA receptor is responsible of cell death in stroke and related hypoxic or ischemic conditions.[166a] The receptor has different binding sites, among them for glycine and for glutamate. Antagonists acting at the glycine binding site appear to have less adverse side effects.[166b–d] Hume and Nagata[142] performed radioligand binding inhibition assays using [$^3$H]-5,7-dichlorokynurenic acid (**213a**).[167] The $K_i$ values of azakynurenic acids **218a** and **218b** toward the glycine site of the NMDA receptor were 0.91 and 0.25 μM, respectively, indicating that the compounds maintained affinity; however, they were substantially less effective than 5,7-dichlorokynurenic acid itself (0.04 μM).

### 5.8. Antifouling activity

Based on the noteworthy observation of absence of fouling on *Aaptos* sponges, and in an effort to highlight the ecological importance of biochemical defenses, Hamann et al. studied aaptamines as potential zebra mussel (*Dreissena polymorpha*) antifoulants.[46] Aaptamine (**1**), isoaaptamine (**2**), and 8,9-bisdemethylaaptamine (**5**) produced $EC_{50}$ values of 24.2, 11.6, and 18.6 μM, respectively, in the zebra mussel assay. In addition, neither aaptamine nor isoaaptamine at concentrations as high as 300 μM, elicited a phytotoxic response toward duckweed (*Lemna pausicostata*) a nontarget organism, in a 7-day exposure. Thus, aaptamine derivatives may act as environmentally benign antifouling alternatives to currently used metal-based paints and preservatives.[168]

A structure–activity relationship analysis showed that the methyl substitution patterns at the heteroatoms, including free hydroxyls and *N*-methyl groups were important for antifouling activity and reduced toxicity. Compounds **2** and **5**, displaying free OH groups were the most active antifouling compounds. However, when compared to aaptamine and **5**, isoaaptamine showed lower toxicity, probably due to the presence of an *N*-methyl group; on the other hand, 1-*N*-methylaaptamine (**175**) and 4-methylaaptamine (**10**) were inactive.

### 5.9. Antidepressant activity

Hamann et al. observed that aaptamine resembles the chemical structure of known antidepressants. The natural product was determined to have significant antidepressant-like activity in the forced swim test in mice, but failed to produce significant results in the tail suspension test. The drug did not show an increase in locomotor behavior, meaning that the decreased immobility time in the forced swim test was not due to hyperactivity associated to the drug.[169] In contrast, isoaaptamine (**2**) increased immobility time and 8,9-bisdemethylaaptamine (**5**) did not show any effect.[47]

## 6. Concluding remarks

The marine environment and the unique natural products contained therein are still a relatively untapped source of opportunities for drug discovery. Marine sponges are a source of many unusual compounds that are generally not been reported from terrestrial sources. The results of the research on aaptamines suggest that further investigations of this class of marine natural products may prove fruitful in mankind's quest for new medicines. An important part of our current pharmacological arsenal is composed by natural products and their derivatives or drugs inspired in natural products. This underscores both, the importance of natural products and the relevance of optimizing natural product structures to improve their desirable pharmacological properties.

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## Biographical sketch

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