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Synthesis and antimalarial evaluation of new N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)-piperazine derivatives

Adina Ryckebusch,[a] Marie-Ange Debreu-Fontaine,[a] Elisabeth Mouray,[b] Philippe Grellier,[b] Christian Sergheraert[a] and Patricia Melnyk[a,*]

[a]*Institut de Biologie et Institut Pasteur de Lille—UMR CNRS 8525—Université de Lille II 1 rue du Professeur Calmette, B.P. 447, 59021 Lille, France*
[b]*USM 0504, ''Biologie fonctionnelle des protozoaires'', Département, ''Régulations, Développement, Diversité Chimique'', Muséum National d'Histoire Naturelle, 61 rue Buffon, 75005 Paris, France*

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Abstract—Synthesis and evaluation of the activity of new N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine derivatives against a chloroquine-resistant strain of *Plasmodium falciparum* are described. Selectivity indices were improved for two compounds versus the lead **1**, the bis-cyclopropylmethyl derivative, thus increasing the therapeutic interest of our family. As our previous studies conducted on the mode of action of our compounds made us hypothesize the existence of original mechanisms and/or original targets, terminal amino derivatives can be considered as promising tools further mechanistical studies, as probes for affinity chromatography.

1. Introduction

Chloroquine (CQ) (Fig. 1) has been one of the two most widely used antimalarial drugs. The success of CQ was mainly due to its outstanding clinical efficacy, and the slow speed at which resistance developed to this drug. But the final arrival of resistance and the alarming spread of CQ-resistant *Plasmodium falciparum* on a global scale created an urgent need for the development of novel antimalarial drugs.[1]

Interest in 4-aminoquinolines is still prevailing[2,3] and several reports[2–6] have shown that simple modifications on the side chain of CQ lead to analogues significantly more potent than CQ against CQ-resistant strains. These results highlighted the possibility to identify useful CQ analogues based on the same molecular mechanism of action: accumulation by weak-base effect in the acidic digestive vacuole (DV) of the parasite and inhibition of hemozoin formation,[7,8] and circumventing the resistance mechanism.[9]

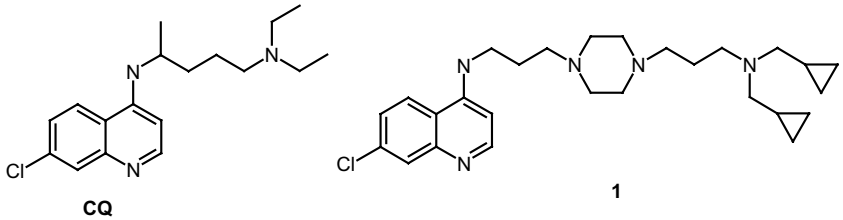

Figure 1. Chloroquine and compound **1**.

Keywords: Antimalarial activity; Quinoline; Piperazine derivatives.
* Corresponding author. Tel.: +33 3 20 87 12 19; fax: +33 3 20 87 12 33; e-mail: patricia.melnyk@ibl.fr

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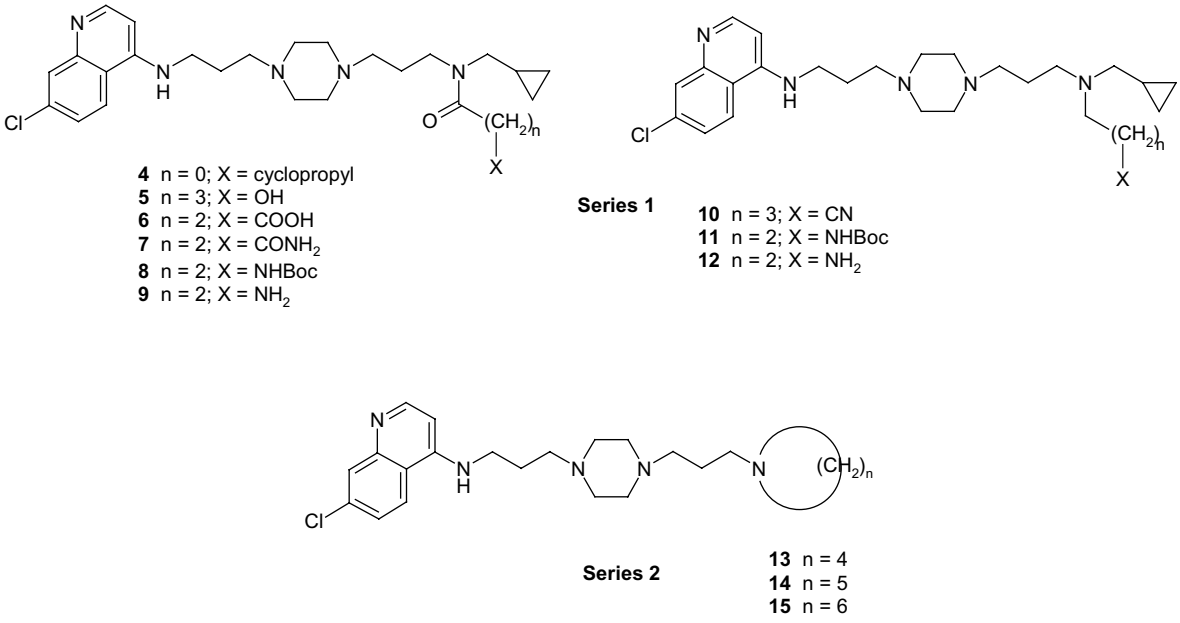


Figure 2. Compounds from Series 1 and 2.

Works in our laboratory focus on new N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine derivatives. A library of sulfonamide derivatives[10] provided compounds up to 100-fold more potent than CQ on the CQ-resistant strain FcB1. More recently[11] we have reported a study concerning three series of N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine amides, secondary amines and tertiary amines. Among them, eleven compounds displayed a higher selectivity index (ratio CC_{50}/IC_{50} activity) than chloroquine (CQ) upon MRC-5 cells, and one of them, compound **1** (Fig. 1), cured mice infected by *Plasmodium berghei*. Despite its curative properties, compound **1** displayed toxicity on treated mice above 20 mg/kg,[12] which hindered its further evaluation as a drug candidate.

Mechanistical studies conducted on our compounds suggested a CQ-like mode of action but also the involvement of additional mechanisms.[11] Fluorescence studies[10] also suggest the existence of additional targets outside the digestive vacuole, possibly involving an original mechanism of action.

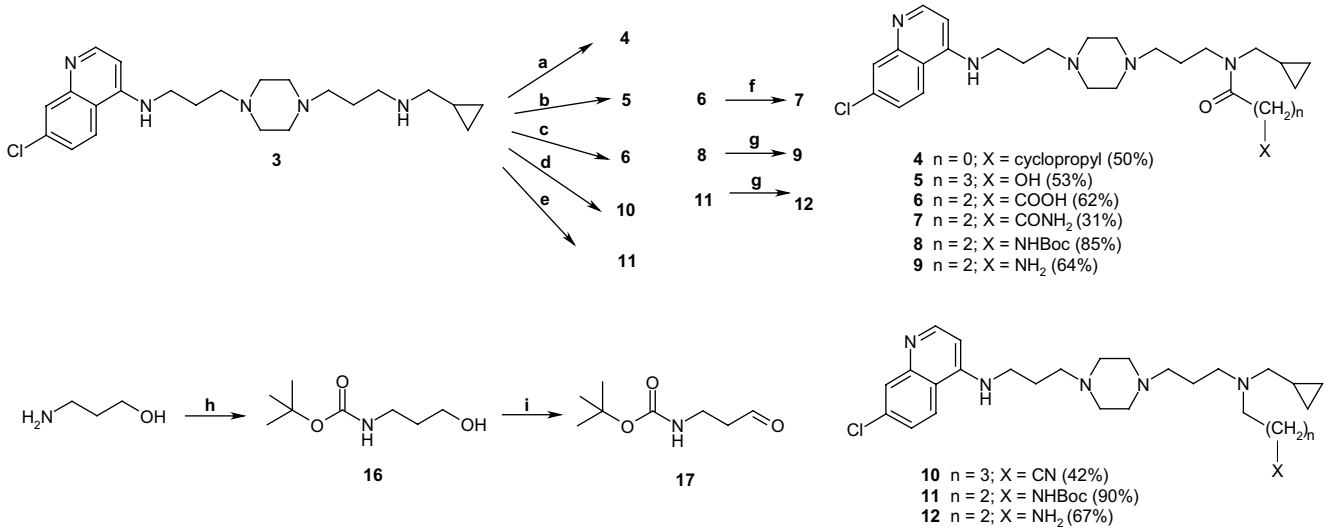
Considering good pharmacological results obtained in this family, and taking into account some previously established structure–activity relationship, we decided to further enlarge chemical diversity in order to get more SAR information in our N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine family and in the hope to improve activity/cytotoxicity ratio (using MRC-5 model). Firstly, considering compound **1** as a lead, we decided to introduce chemical diversity at the place of one of the two methylenecyclopropyl moieties (Series 1, Fig. 2). In addition, for the design of our compounds, we kept in mind their possible fixation on solid support for a ulterior use as pharmacological tools in affinity chromatography biological studies. Secondly, taking into account high antimalarial activities obtained for tertiary amines, in our previous series (among whom compound **1**) we decided to replace both methylenecyclopropyl moieties by more constraint entities (Series 2, Fig. 2).

2. Chemistry

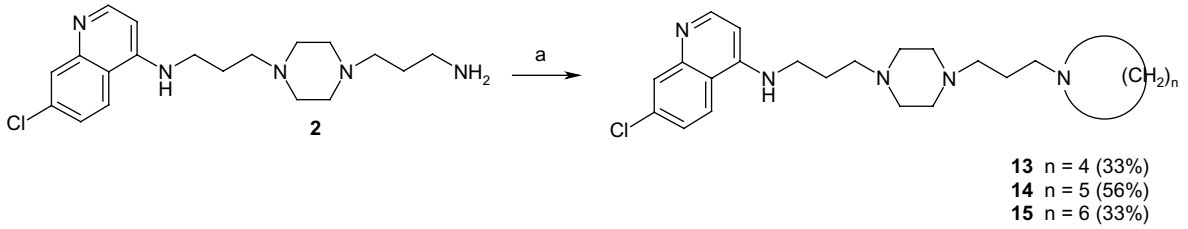
Compound **3** obtained by a procedure described previously,[11] was used as a precursor for synthesis of compounds **4**–**12** (Scheme 1). Amides **4** and **8** were prepared by reaction of amine **3** with respectively cyclopropyl carboxylic acid and Boc-β-Ala, using HBTU and HOBT as coupling agents and DIEA as a base. Amides **5** and **6** were obtained respectively by reaction with γ-butyrolactone and succinic anhydride. Synthesis of tertiary amine **11** was achieved by reductive amination from aldehyde **15** and amine **3** using sodium acetoxyborohydride. Compound **15** was previously synthesized by Boc-protection of commercially available 3-amino-propan-1-ol, followed by oxidation of alcohol to aldehyde with pyridinium chlorochromate. Primary amines **9** and **12** were obtained by deprotection of compounds **8** and **11**. The succinamide **7** was prepared from acid **6**, di-*tert*-butyl dicarbonate and ammonium carbonate.

Synthesis of cyclic amines **13**–**15** was achieved by nucleophilic substitution of primary amine **2**, previously synthesized, with respectively dibromobutane, dibromopentane and dibromohexane (Scheme 2).[13]

The compounds were characterized by MALDI-TOF and NMR and the data were consistent with the structure.[14] The purity of final compounds was assumed by high-pressure liquid chromatography (HPLC).[15]



Scheme 1. Synthesis of compounds **4–12** (Series 1). Reagents and conditions: (a) appropriate carboxylic acid, DIEA, HBTU, HOBT, CH_2Cl_2, rt, 4 h, 50–85%; (b) γ-butyrolactone, dry CH_3CN, 80 °C, 48 h, 53%; (c) succinic anhydride, dry CH_3CN, 80 °C, 4 h, 62%; (d) 5-chlorovaleronitrile, N-ethylpiperidine, CH_3CN, 40 °C, 6 h, 42%; (e) compound **17**, $NaHB(OAc)_3$, CH_2Cl_2, rt, 4 h then NaOH, 90%; (f) Boc_2O, TEA, distilled THF, 0 °C, 1 h then $(NH_4)_2CO_3$, rt, 16 h, 50%; (g) TFA/CH_2Cl_2: 1/1, rt, 2 h, 74–75%; (h) Boc_2O, dioxane/0.5 N NaOH: 1/1, 16 h, 80%; (i) pyridinium chlorochromate, dry CH_2Cl_2, rt, 4 h, 90%.



Scheme 2. Synthesis of compounds **13–15** (Series 2). Reagents and conditions: (a) appropriated dibromoalcane, DMF, K_2CO_3, rt, 48 h, 33–56%

3. Biological evaluation

The antimalarial activities of the two series of compounds were determined by their inhibition of parasite growth using the CQ-resistant strain FcB1 (IC_{50}(CQ) = 126 nM).[16,17] Results are given in Table 1.

In parallel, all compounds were tested for cytotoxicity upon a human diploid embryonic lung cell line (MRC-5 cells) using the colorimetric MTT assay.[18]

4. Results and discussion

In Series 1 the introduction of a variety of fragments by means of amide and tertiary amine links provided compounds with a broad range of antimalarial activities.

The highest antimalarial activities were obtained for compounds **4**, **10** and **11** which exhibited low IC_{50} values between 6.5 and 12.6 nM, up to 20-fold lower than CQ.

Replacement of the methylenecyclopropyl moiety in compound **1** by propylene–carbamic acid *tert*-butyl ester (compound **11**) or butylcyanide (compound **10**) provided compounds with activities 6-fold better than CQ and similar to that of compounds **1** and **3**, suggesting that rather bulky and hydrophobic substituents can be introduced in this position without losing activity.

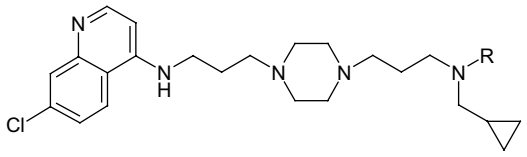
Boc-protection of the terminal primary amine in compounds **8** and **11** enhance considerably the antimalarial activity when compared to their free amine counterparts, compounds **9** and **12**.

Introduction of R substituent by means of an amine link instead of amide led to more effective inhibition in the case of derivatives containing a terminal primary amine, free or protected by a Boc group. Indeed, activities of the amine compounds **11** and **12** were found to be more than 2-fold superior to those of their respective counterparts amides **8** and **9**.

The average cytotoxicities of our compounds in Series 1 upon MRC-5 cells extended from 4 μM to more than 32 μM. This range provided two selectivity indices (compounds **4** and **10)** superior to that of CQ, the selectivity of compound **4** being 5-fold superior to that of reference compound **1**.

In Series 2, introduction of cyclic tertiary amines provided compounds between 6- and 11-fold more potent

Table 1.



No.	R	IC_{50} of parasite growth (nM)[a]	CC_{50} (μM)[b]	Selectivity index (CC_{50}/IC_{50})
	CQ	126 (±26)	50	397
Series 1				
1[c]	~CH$_2$—cyclopropyl	8.8 (±1.7)	4.0	454
3[c]	~H	10.2 (±7.6)	7.6	745
4	~CO—cyclopropyl	6.5 (±2.3)	13.5	2076
5	~CO—(CH$_2$)$_3$—OH	172 (±9.2)	21	122
6	~CO—(CH$_2$)$_2$—COOH	1380 (±50)	>32	>23
7	~CO—(CH$_2$)$_2$—CONH$_2$	<1000	>32	Nd[d]
8	~CO—(CH$_2$)$_2$—NHBoc	34.2 (±3.8)	4	116
9	~CO—(CH$_2$)$_2$—NH$_2$	398 (±56)	>32	>80
10	~(CH$_2$)$_4$—CN	9.9 (±0.2)	4	404
11	~(CH$_2$)$_3$—NHBoc	12.6 (±4.4)	4	317
12	~(CH$_2$)$_3$—NH$_2$	152 (±8)	>32	>210
Series 2				
13	~N (pyrrolidine)	19.6 (±1.0)	20	1020
14	~N (piperidine)	11.6 (±2.4)	5.1	439
15	~N (azepane)	13.4 (±4.3)	5.0	373

[a] IC_{50} values were obtained from triplicate experiments performed on the FcB1 strain. Standard error is given in brackets.
[b] MRC-5 cells (human diploid embryonic lung cell line).
[c] See Ref. 11.
[d] Nd: not determined.

than CQ. Shortening the heterocycle induced a slight decrease in activities but concomitantly lower cytotoxicities (compound **13** vs compounds **14**, **15**), leading for compound **13** to the best selectivity index in this series (2-fold better than compound **1**).

Comparison of compound **13** with our previous results concerning its diethylamine counterpart[11] showed that rigidification in this region of the molecule yielded a slight loss in activity but also a 200-fold decrease in cytotoxicity, which finally provided for compound **13** a 22-fold increase in the selectivity index.

Further in vivo studies are going to be conducted on compounds **4** and **13** displaying selectivity indices superior to that of reference compound **1**.

Our previous study[11] conducted on 69 derivatives displaying a common N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine moiety showed that a selection of 19 representative compounds displayed high in vitro inhibition of β-hematin (equivalent of hemozoin) formation. Replacement of the quinoline moiety in one of these compounds by a series of heterocycles[19] led to a loss of the ability to inhibit β-hematin formation (with the exception of benzimidazole). Inhibition of β-hematin formation by our compounds appeared thus to be essentially due to the presence of the 7-chloro-4-aminoquinoline nucleus, consistent with studies conducted inside the DV on CQ and derivatives[20,21] that have demonstrated that the 7-chloro-4-amino-quinoline nucleus is responsible for hematin binding, inhibition of hemozoin formation and antimalarial activity, and that the side chain contributes only minimally to hematin binding. In order to assess the role of accumulation in the antimalarial activity we calculated the free intravacuolar accumulation ratio (VAR),[22] corresponding to equilibrium in the distribution of free compound. Even if our

compounds display generally good inhibition of hematin formation and high intravacuolar accumulation by weak-base character, no direct correlation could be established between these parameters and antimalarial activity. Exceptions such as aromatic carboxamide derivatives made us suppose the existence of additional mechanisms. Fluorescence studies conducted on sulfonamide derivatives[10] made us assume that some of our compounds could have additional targets outside the digestive vacuole, possibly involving an original mechanism of action. The next step will consist in the search of these putative biological targets by the affinity chromatography technique, using a derivative of compound **1**. Compounds **9** or **12** can be considered as good candidates for this technique, as their respective amino terminal function enable fixation on solid support while preserving significant antimalarial activities (respective IC_{50} 398 nM and 152 nM).

5. Conclusion

This work provided us with additional structure–activity and structure–cytotoxicity information in the N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine family. Indeed, this study proved that a number of substitutions lead to compounds with high activities and reduced cytotoxicities.

Synthesis and evaluation of the compounds in Series 1 showed that lipophilic substituents could be introduced in the place of one of the cyclopropyl methylene moieties in compound **1** while maintaining a high antimalarial activity. Introduction of cyclic tertiary amines on the N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine template provided three additional compounds with high antimalarial activities, among which one displayed improved selectivity index compared with reference compound **1**.

Further investigation is required in order to enlarge diversity on the second methylenecyclopropyl member in compound **1**. Parallel synthesis of two libraries of amides and tertiary amines compounds are being undertaken using compound **3** as a common precursor. Taking into account our first structure–activity relationship, these libraries will include on the terminal region of the molecule derivatives of primary amines and alcohols (i.e., etheroxides, esters, secondary and tertiary amines and amides, carbamic acid esters) and derivatives of carboxylic and hydroxamic acids.

Acknowledgements

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12. Two mice out of three died owing to toxicity at 40 mg/kg of compound **1**.
13. General procedure of the preparation of cyclic amines **13**–**15**: To a solution of amine **2** (150 mg, 0.41 mmol) in 5 mL of DMF were added the appropriated dibromoalcane (1.2 equiv) and K_2CO_3 (287 mg, 5 equiv). After the mixture was stirred at room temperature for 48 h, dichloromethane was added and the mixture was washed with aqueous 1 M $NaHCO_3$. The organic layer was separated and dried over $MgSO_4$, the solvent was evaporated and the residue was purified by thick layer chromatography to yield the desired product.
14. ^1H and NMR spectra were obtained using a Bruker 300 MHz spectrometer. Chemical shifts (δ) were expressed in ppm relative to TMS used as an internal standard. Mass spectra were recorded on a time-of-flight (TOF) plasma desorption spectrometer using a californium source or on a MALDI-TOF Voyager-DE-STR spectrometer. Examples are given for compounds **4** and **13**.
4 ^1H NMR ($CDCl_3$), δ (ppm): 8.35 (d, J = 5.4 Hz, 1H, Ar–H), 8.27–8.20 (m, 1H, Ar–H), 8.01 (d, J = 1.9 Hz, 1H, Ar–H), 7.39–7.34 (m, 1H, Ar–H), 6.39 (d, J = 5.5 Hz, 1H, Ar–H), 3.49–3.45 (m, 4H, CH_2), 3.30–3.27 (m, 2H, CH_2), 2.80–2.75 (m, 10H, CH_2), 2.55–2.51 (m, 2H, CH_2), 2.07–2.05 (m, 2H, CH_2), 1.98–1.62 (m, 3H, CH and CH_2), 0.96–0.79 (m, 5H, CH and CH_2), 0.53–0.48 (m, 2H, CH_2), 0.27–0.23 (m, 2H, CH_2); TOFMS m/z = 484.4.
13 ^1H NMR ($CDCl_3$), δ (ppm): 8.42 (d, J = 5.4 Hz, 1H, Ar–H), 7.86 (d, J = 2.1 Hz, 1H, Ar–H), 7.80 (d, J = 8.9 Hz, 1H, Ar–H), 6.24 (d, J = 5.4 Hz, 1H, Ar–H), 3.33–3.27 (m, 2H, CH_2), 2.58–2.40 (m, 18H, CH_2), 1.93–1.83 (m, 2H, CH_2), 1.79–1.68 (m, 6H, CH_2); MALDI-MS m/z = 416.2.
15. Analytical HPLC was performed on a Shimadzu system equipped with a UV detector set at 254 nm. HPLC column: C18 nucleosil using the following eluent system: A (H_2O/TFA:100/0.05) and B (CH_3CN/H_2O/TFA: 80/20/0.05). HPLC retention times (HPLC t_R) were obtained at

flow rates of 1 mL/min using the following conditions: a gradient run from 100% eluent A for 1 min, then to 100% eluent B over the next 30 min.**4**: $P_{HPLC} < 99\%$, t_R 18.41 min; **5**: $P_{HPLC} < 99\%$, t_R 12.98 min, MALDI-MS $m/z = 502.3$; **6**: $P_{HPLC} < 99\%$, t_R 13.42 min, MALDI-MS $m/z = 516.3$; **7**: P_{HPLC} 93%, t_R 20.74 min, MALDI-MS $m/z = 515.3$; **8**: $P_{HPLC} < 99\%$, t_R 16.55 min, MALDI-MS $m/z = 587.2$; **9**: $P_{HPLC} < 99\%$, t_R 11.83 min, MALDI-MS $m/z = 487.6$; **10**: $P_{HPLC} < 99\%$, t_R 12.72 min, MALDI-MS $m/z = 497.5$; **11**: $P_{HPLC} < 99\%$, t_R 14.97 min, TOFMS $m/z = 573.3$; **12**: $P_{HPLC} < 99\%$, t_R 11.31 min, TOFMS $m/z = 473.3$; **13**: $P_{HPLC} < 99\%$, t_R 11.41 min; **14**: $P_{HPLC} < 99\%$, t_R 11.67 min, MALDI-MS $m/z = 430.2$; **15**: P_{HPLC} 95%, t_R 12.26 min, MALDI-MS $m/z = 444.1$.

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