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Armeniaspiroles, a new class of antibacterials: antibacterial activities and total synthesis of 5-chloro-Armeniaspirole A

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ABSTRACT

Armeniaspiroles, a novel class of natural products isolated from *Streptomyces armeniacus*, are characterized by a novel spiro[4.4]non-8-ene scaffold. Various derivatives of Armeniaspiroles could be obtained by halogenation, alkylation, addition/elimination or reductions. A total synthesis of the 5-chloro analog of Armeniaspirole A has been accomplished in a linear six-step sequence. 5-Chloro-Armeniaspirole A exhibits good activity against a range of multidrug-resistant, Gram-positive bacterial pathogens.

Infectious diseases are one the leading causes of death worldwide. The growing incidence of bacterial resistance to the present antibacterials represents a serious medical challenge.[1,2] Multidrug-resistant Gram-positive pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermis* (MRSE), vancomycin-resistant *Enterococci* (VRE) and cephalosporin-resistant *Streptococcus pneumonias* are responsible for significant morbidity and mortality of infected patients.[3,4] The discovery and development of new antibacterials without cross-resistance to current drugs has therefore become an urgent task. Despite great efforts from academic institutes and pharmaceutical companies, only two drugs that represent novel chemotypes have been introduced to the market in the last decade, that is, linezolid and daptomycin.[5,6]

In order to address the increasing medical need, we have investigated bacterial microorganisms, a superior source of new antibiotic drugs, to identify novel scaffolds with antibacterial activity. The cultivation of *Streptomyces armeniacus* under specific conditions led to the discovery of Armeniaspiroles, a new class of compounds biosynthetically related to Streptopyrroles.[7,8]

The structures of the Armeniaspiroles were elucidated by NMR spectroscopy and X-ray[9], thereby establishing the relative and absolute configuration. Armeniaspiroles A (**1**), B (**2**) and C (**3**) exhibit an unprecedented 1-oxa-6-aza-spiro[4.4]non-8-ene scaffold; they differ by their alkyl chain that is linear in **1** and branched in **2** and **3** (Fig. 1). Armeniaspiroles were isolated as the enantiopure *R* isomers at the spiro stereocenter; **3** contains a second stereocenter of *S* configuration.

Subsequent growth inhibition screening against bacteria led to the discovery of the antibacterial properties of **1–3**, qualifying Armeniaspiroles as novel antibacterial lead structures.

In this Letter, we report our efforts to establish both semisynthetic and total synthetic routes towards the spiro[4.4]non-8-ene scaffold of Armeniaspiroles in order to explore the structure–activity relationships. Besides, additional biological data on the natural compounds and their analogs are reported.

First, a chemical derivation program of the natural compounds was initiated. Schemes 1 and 2 exemplify some derivatives of **1** and **3**, respectively, which were obtained after probing the reactivity with selected electrophiles, nucleophiles and reducing agents. These chemical modifications demonstrates first preliminary

Figure 1. Structure of Armeniaspiroles A, B, C and semisynthetic 5-chloro-Armeniaspirole A derivative.

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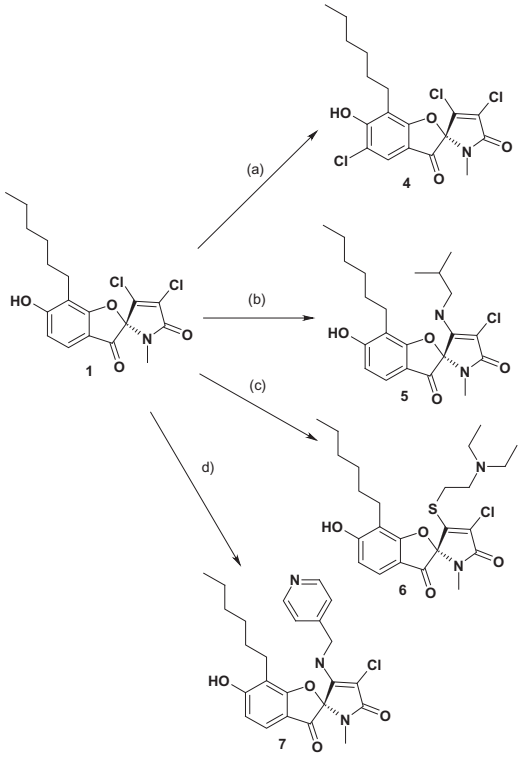
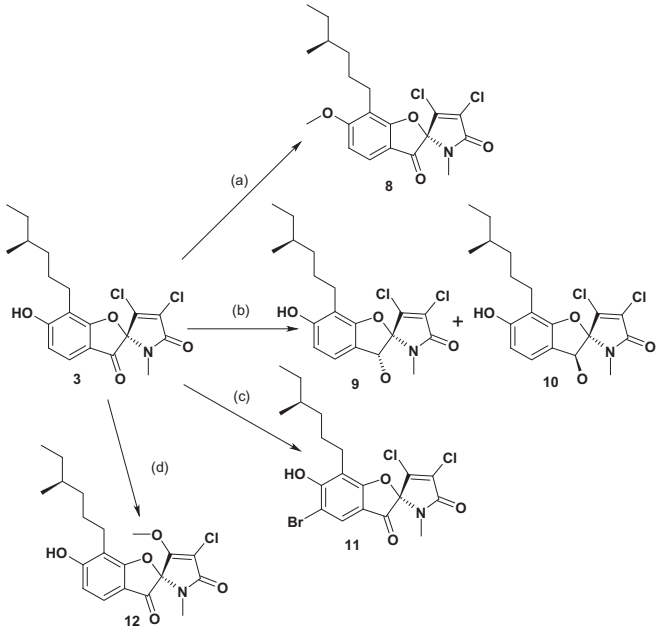


Scheme 1. Armeniaspirol A derivatives. Reagents and conditions: (a) SO_2Cl_2 (1.2 equiv), CH_2Cl_2, rt, 12 h, 72%; (b) isopropyl amine (2 equiv), NEt_3 (2 equiv), CH_2Cl_2, 50 °C, 12 h, 61%; (c) 2-diethylamino-ethanethiol·HCl (2 equiv), NaH (2 equiv), CH_2Cl_2, 50 °C, 12 h, 65%; (d) pyridin-4-yl-methylamine, (2 equiv), NEt_3 (2 equiv), CH_2Cl_2, 50 °C, 12 h, 73%.



Scheme 2. Armeniaspirole C derivatives. Reagents and conditions: (a) MeI (5 equiv), K_2CO_3 (5 equiv), DMF, 40 °C, 3 h, quant; (b) excess $BH_3{\cdot}^tBuNH_2$,THF/MeCN (9:1), 60 °C, 10 h, 49% of **9** and 13% of **10**; (c) Br_2 (1.2 equiv), AcOH, RT, 12 h, 68% (d) NaOMe (10 equiv), MeOH, 80 °C, 12 h, 18%.

structure activity relationship and did not show any improved antibacterial activities except with the incorporation of halogen into the phenol group.

Halogenation of the Armeniaspiroles with Br_2 or SO_2Cl_2 furnished selectively the *ortho*-substituted derivatives (relative to the phenolic OH function) **4** and **11**. The reaction of **1** with the *N*-nucleophiles isopropyl amine and pyridine-4-yl-methylamine, and the *S*-nucleophile 2-diethylamino–ethanethiol selectively led to nucleophilic substitution products **5**, **7**, and **6** at the activated β-position of the unsaturated lactame. A substitution by *O*-nucleophiles was hardly feasible; the less nucleophilic reagent NaOMe reacted with **3** under more drastic conditions to give the corresponding methoxy substitution product **12** in low (18%) yield.

Alkylation of the phenol function could be achieved in good to excellent yields with alkyl bromides or alkyl iodides in dimethylformamide in the presence of K_2CO_3. The ketone moiety of the furan ring could be reduced chemoselectively with excess $BH_3{\cdot}^tBuNH_2$ to an 8:2 mixture of the corresponding secondary alcohols **9** and **10**.

In order to get access to broader structural variations, a total synthesis of the Armeniaspiroles was envisaged. In 1999, Raggatt et al.,[10] reported a study on the biosynthesis of streptopyrrole (XR857) **17**, a secondary metabolite isolated from two *Streptomyces sp*. with a pyrrolobenzoxazin skeleton (Scheme 3). Isotope labeling studies established a biosynthetic pathway through a sequence of oxidation and chlorination steps with a pyrrol-polyketide intermediate **13**, closely related to the pyrrolomycin-type metabolites from *Streptomyces fumans*,[11] and a postulated *spiro*-intermediate **15**, which represents essentially the 1-oxa-6-aza-spiro[4.4]non-8-ene skeleton of the Armeniaspiroles. Although intermediates **13** and **15** were neither isolated nor characterized, Scheme 3 indicates the close biosynthetic relations between Streptopyrrole, the Pyrrolomycins and the Armeniaspirols.

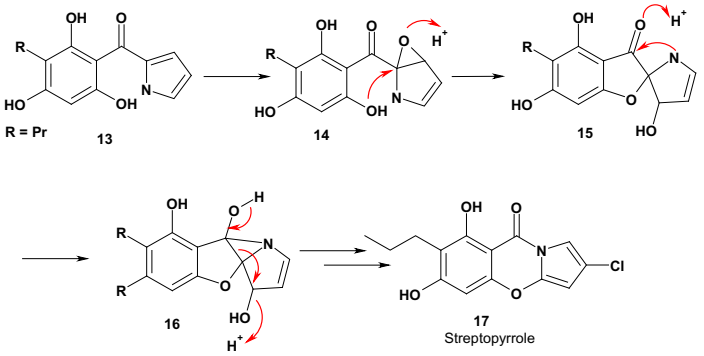
Inspired by this biosynthetic pathway, a biomimetic synthesis to the Armeniaspirole core was envisaged starting from **19** (Scheme 4). The pyrrolo-phenone precursor **19** was readily available in two steps from *N*-methyl pyrrole and 2-methoxy-benzoyl chloride under modified Friedel–Crafts conditions, followed by Lewis acid cleavage of the methyl ether.

In first attempts to achieve a *spiro*-cyclisation intermediate, **19** was subjected to oxidative halogenation conditions with 2 equiv of bromine in acetic acid at room temperature. However, no cyclisation was observed. Instead, di-bromination of the pyrrole nucleus led to a simplified analogue **20** of the pyrrolomycins in 71% yield. Treatment of **20** with trifluoroacetic acid at room temperature gave a mixture of products (**21** and **22**) with a pyrrolobenzoxazin skeleton that is found in TAN-876A, a metabolite from *Streptomyces sp.* C-70899.[12] The reaction **20** with N-bromosuccinimide in acetic acid at 70 °C finally led to a mixture of mono-, di- and tribrominated products **23**, **24** and **25** with the desired *spiro*-skeleton, the main product being the di-bromo derivative.

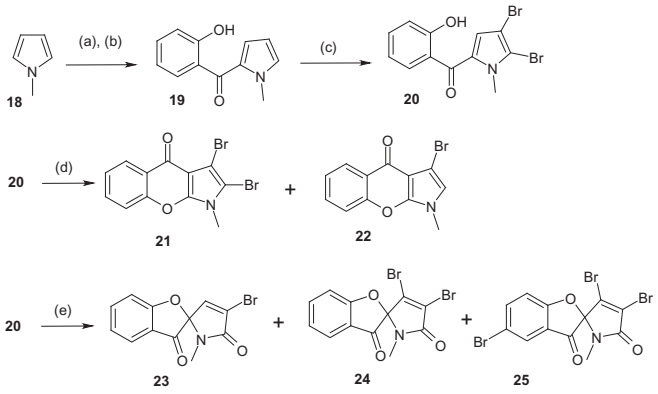
Reaction of **20** with N-chlorosuccinimide in acetic acid under optimized conditions gave the trichlorinated *spiro*-intermediate **27** as a main product in 65% yield (Scheme 5). A dehydrohalogenation with NEt_3 in $CHCl_3$ furnished **30**, a compound with the correct racemic Armeniaspirole skeleton, in 92% yield.

A refined approach to Armeniaspiroles bearing the alkyl chain substitutions is summarized in Scheme 6. We envisioned to construct the highly functionalized tricyclic scaffold starting from 2,6-dimethoxybromobenzene. The alkyl chain was attached through a Suzuki-Miuyra coupling[13] with the corresponding hexylboronic acid that proceeded in 70% yield to afford compound **31**. Friedel–Crafts reaction of the latter with pyrrole-2-carbonyl chloride **32** provided compound **33** in a moderate 60% yield.

The next step of the sequence comprised a selective demethylation with BBr_3 to give compound **34** (82% yield). The later was engaged in the key cyclisation step. After consecutive treatment of **34** with N-chlorosuccinimide in acetic acid and triethylamine, com-



Scheme 3. Biosynthesis of streptopyrrole **17** according to Ref. 9.



Scheme 4. Reagents and conditions: (a) 2-Methoxy-benzoyl chloride (1.5 equiv), $Yb(OTf)_3$ (1 equiv), [bpy][BF_4], rt, 2 h, 92% (b) $BBr_3{\cdot}Me_2S$ (2 equiv), $(CH_2Cl)_2$, 80 °C, 2 h, quant. (c) Br_2 (2.2 equiv), rt, 2 h, 71% (d) TFA, rt, 2 h, 46% of **21** and 39% of **22** (d) NBS (2 equiv), AcOH, 70 °C, 4% of **23**, 28% of **24** and 14% of **25**.

pound **35** was obtained in 56% yield. The construction of the *spiro*-cycle was accompanied by an unintended chlorination of the aromatic ring. Unfortunately, all attempts to synthesize Armeniaspirol without a chlorinated phenol ring failed. The synthesis of 5-Chloro-Armeniaspirole A was completed by N-methylation of the lactam and deprotection of the phenol by BBr_3 to afford compound **36** in 72% yield. The two enantiomers of racemic 5-Chloro-Armeniaspirole A **36** were finally separated by chiral chromatography to give enantiopure 5-Chloro Armeniaspirole A **4** (*R*-configuration) and **37** (*S*-configuration). The analytical data of synthetic, *R*-configured 5-Chloro-Armeniaspirole A matched those of the semisynthetic product obtained according to Scheme 1.

Naturally occurring Armeniaspiroles and the semisynthetic derivatives **4**, **36** and **37** display good in vitro activities with IC_{80} values in the low μM range against Gram-positive bacteria, including Methicillin-resistant or sensitive *Staphylococcus aureus* (MRSA, MSSA), penicilline-resistant *Streptococcus pneumoniae* (PRSP), or vancomycin resistant *Enterococcus faecium* (VRE) (Table 1).

In vitro studies also reveal that incorporation of a chlorine atom onto the phenol ring reduces the antibacterial activity of **4** compared to natural **1** but increases metabolic stability. Surprisingly, there is no significant difference in activity between the enantiomers **4** and **37**, and racemic **36** on the other hand.

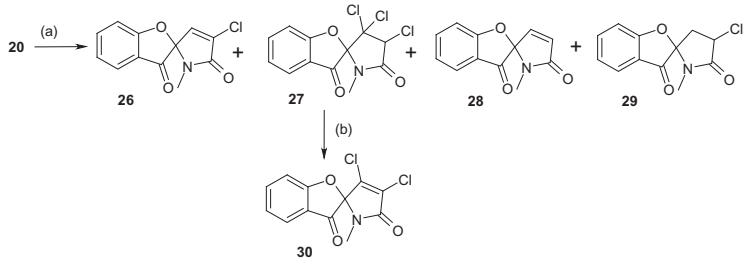
The natural members of the Armeniaspirole family exhibited low solubilities of 25, 10 and 5 μg/ml for **1**, **2** and **3**, respectively, at pH 7.4 in PBS buffer. The compounds are fully stable over 24 h under these conditions. Incubation of **1** with either 10 mM GSH or 10 mM *i*-$BuNH_2$ in PBS buffer led to a decrease of the parent compound's concentration by 30% or <10% over 24 h, respectively. Thus, the chemical stability of **1** towards *S*- and *N*-nucleophiles is sufficient, despite its potentially reactive β-chloro-Enone moiety.

Time-kill studies indicated that Armeniaspiroles are bacteriostatic with a time-dependent effect. No sign of resistance development against Armeniaspirole after 48 h at concentration >1 μg/ml could be detected. In vitro studies indicated also that activity decreased in the presence of fetal calf serum (data not shown).

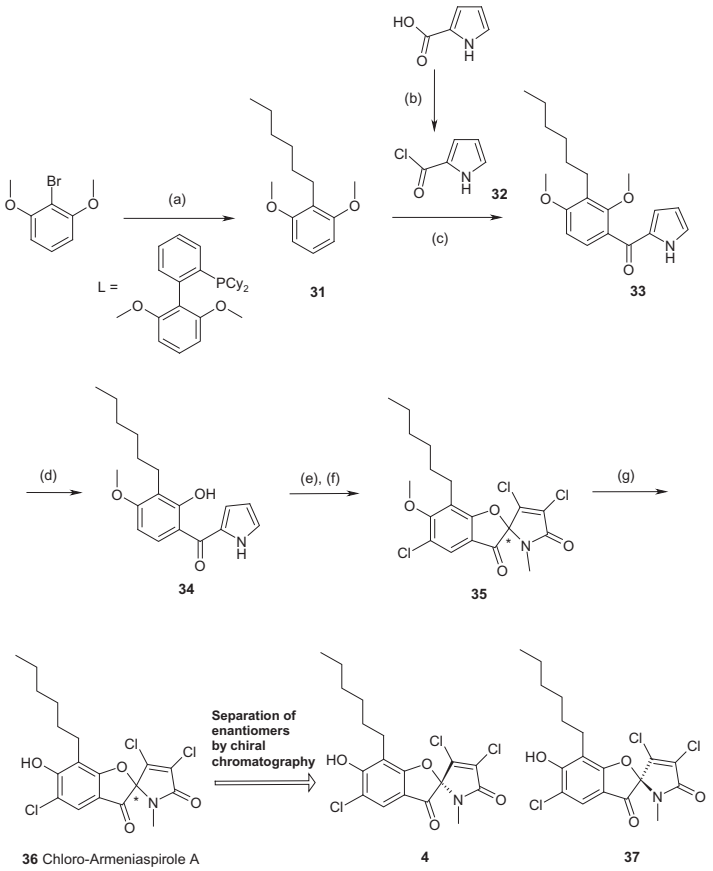
Compound **1** exhibited no strong interaction with cytochromes CYP3A4 and CYP2D6 (IC_{50} > 10 μM), and no activity on the hERG cardiac channel (inhibition < 35% @ 10 μM). In a PK study in male Swiss mice, (7.5 mg/kg by iv route), **1** exhibited a moderate clearance (1.2 L/h/kg), a large volume of distribution (*V*ss = 2.4 L/kg), a c_{max} of 27.4 μg/ml, and a moderate half-life in plasma ($t_{1/2}$ = 3 h).

In a previous report, in vivo studies in an MRSA-induced septicemia model (Table 2) demonstrated that **1** improved dose-dependently survival and dose-dependently decreased blood bacteremia in MRSA-infected mice following iv administration.

In this study, an identical experimental setup was chosen in order to assess the efficacy after sc administration of 20, 40, and 80 mg/kg. Again, significant effects on survival and on the reduction of blood bacteremia levels were observed. However, higher



Scheme 5. Reagents and conditions: (a) NCS (2.2 equiv), AcOH, rt, 1 h, then another 2 equiv of NCS, 70 °C, 2 h (one pot) 9% of **26**, 65% of **27**, 8% of **28** and 1% of **29** (b) NEt_3 (2 equiv), $CHCl_3$, 50 °C, 2 h, 92%.



Scheme 6. Total synthesis of 5-chloro-Armeniaspirol. Reagents and conditions: (a) $CH_3(CH_2)_5B(OH)_2$, $Pd(OAc)_2$, Ligand L, $K_3PO_4{\cdot}H_2O$, toluene, 100 °C, 70%; (b) $(COCl)_2$, DMF cat., CH_2Cl_2, 100%; (c) $SnCl_4$, CH_2Cl_2, 0 °C, 60%; (d) BBr_3, $(CH_2Cl)_2$, 82%; (e) NCS, AcOH, 70 °C then Et_3N, CH_2Cl_2, 60 °C, 56%; (f) CH_3I, NaH, DMF; (g) BBr_3, $(CH_2Cl)_2$ (72% for 2 steps).

Table 1
In vitro activities: growth inhibition (IC_{80}, μM)

Activity	MRSA (ATCC 33592)	PRSP (DSM 11865)	VRE (DSM 17050)	*E. coli*-LacR (ATCC 35218)	*P. aeruginosa* (ATCC 27853)
1	0.125	2.3	8.2	>30	>30
4 (Semisynth)	5.1	1.7	8.5	>30	>30
36	3.1	1.3	5.2	>30	>30
4 (Synth)	3.7	0.4	8.2	>30	>30
37	1.4	0.5	6.7	>30	>30
Vancomycin	0.4	0.2	>30	nt	nt

MRSA or MSSA = methicillin-resistant or –sensitive; *Staphylococcus aureus*; PRSP = penicillin-resistant *Streptococcus Pneumoniae*; VRE = vancomycin resistant *Enterococcus Faecium*; n.t. = not tested.

Table 2
*I*n vivo activities in a *MRSA*-induced septicemia model

MRSA (ATCC33592)	1 (sc route)			1 (iv route)			Vancomycin	
Dose (mg/kg/d)	20	40	80	3	10	15	40 (sc)	20 (iv)
% Survival	0	50	50°	0	33	67	100°°°	100°°°
Log reduction bacteremia	0.2	0.8°°°	1.7°°°	0.05	0.9°°°	2.8°°°	4°°°	4.2°°°

Results are expressed as Mean ± sem.
(Six animals/group for survival, five animals/group for bacteremia).
$P < 0.05$ or $P < 0.001$ vs vehicle-treated group.
(Vehicle: Ethanol 5%/Solutol 5%/Tris–NaCl buffer pH 9).

doses were required compared to the iv route, and even at the highest tested dose of 80 mg/kg, a complete curation could not be achieved.

Armeniaspiroles constitute a new class of antibacterial natural products. In this study, we have generated various analogs of the natural products though semisynthesis. In addition, a first syn-

thetic access to the spiro[4.4]non-8-ene scaffold of Armeniaspirols could be established. Racemic 5-Chloro-Armeniaspirole A has been synthesized through a linear six-step sequence from readily accessible starting materials. The analog retained the good in vitro activity against Gram-positive bacteria and invites to further explore the SAR of Armeniaspirols.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.107.

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