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Total Synthesis of a Noricumazole A Library and Evaluation of HCV Inhibition

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Dedicated to Professor Dr. Dr. h.c. Ekkehard Winterfeldt on the occasion of his 80th birthday

Abstract: The total synthesis of 16 new ion channel inhibitors derived from noricumazole A, a secondary metabolite from the myxobacterium *Sorangium cellulosum*, is reported. Particular focus of library design is put on stereochemical permutations in the central region (C9 and C11), the oxazole moiety and the side chain at C4 of the isochromanone moiety. Noricumazole A and all new noricumazole derivatives were tested in an assay system with inhibitory effect on the hepatitis C virus (HCV) life cycle. Most of them are moderate to strong HCV inhibitors (350 nM–6 nM) but also exert pronounced cytotoxicity. In contrast, the thiazole analogue of noricumazole A is a strong HCV inhibitor with only moderate cytotoxic property. It may become a lead structure with a good therapeutic index (CC_{50}/IC_{50}) of greater than 10.

Keywords: compound library · Hepatitis C virus · iron catalysis · natural products · total synthesis

Introduction

Recently, noricumazole A (**1a**) and the related icumazole A (**2a**) were isolated from the myxobacterium *Sorangium cellulosum* and the structures including all stereogenic centers were elucidated by spectroscopic methods, chemical derivatization, controlled degradation and total synthesis (Figure 1).[1] Other members of these secondary metabolites, noricumazole B (**1b**) and noricumazole C (**1c**) include glycosylated derivatives at C11 and C18, respectively. Glycosylated derivatives of icumazole A (**2a**), namely icumazole B1 (**2b**) and B2 (**2c**) are also known.[1] Remarkably, noricumazole A (**1a**) is the first natural product reported that shows a stabilizing effect on the tetrameric architecture of the potassium channel KcsA thereby blocking it (1–4 μM).[1]

In addition, noricumazole A (**1a**) was found to interfere with the complete life cycle of the hepatitis C virus (HCV) in cell culture while exerting only moderate cytotoxicity towards the host cell.[2] HCV is one of the primary causes of chronic liver disease worldwide. In fact, chronic HCV infection is associated with chronically active hepatitis, fibrosis, and often results in end-stage liver cirrhosis and hepatocellular carcinoma. Although treatment options have been considerably improved over the past decades, and the first directly acting antivirals were licensed in 2011, side effects, drug resistance and viral genotype-specific differences in efficacy of these therapies still limit current treatment options. Thus, novel therapies are needed to efficiently treat the about 160 million chronically infected HCV patients worldwide.

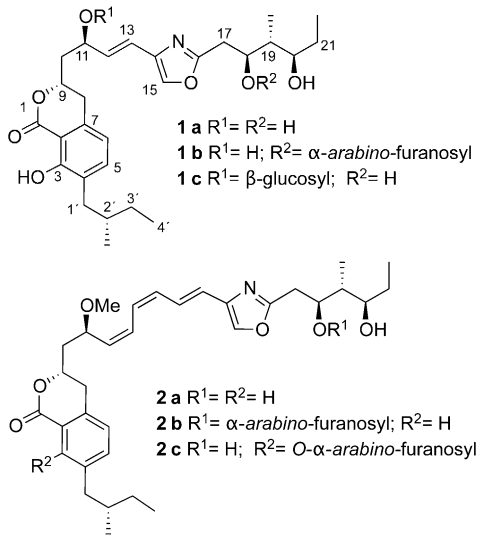

Figure 1. Noricumazoles A–C (**1a–c**) and icumazoles A–C (**2a–c**); numbering does not relate to IUPAC; however, for clarity the depicted numbering was chosen in this report.

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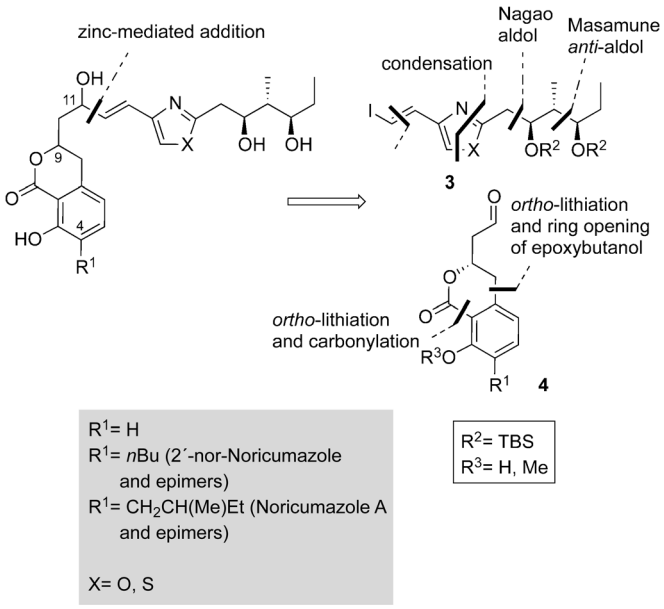
This preliminary finding initiated a total synthesis program in our laboratories to generate a library of noricumazole A derivatives and carry out a first study on the structure–activity relationship. The synthesis program is based on our first total synthesis of noricumazole A (**1**)[1] that helped to unequivocally elucidate the relative and absolute configurations of all stereogenic centers. The program was aimed at simplifying noricumazole A, specifically with respect to the unusual 2-methylbutyl side chain bound at C4 of the aromatic moiety. Furthermore, we envisaged the permutation of the stereogenic centers at C9 and C11, respectively, as well as the exchange of the oxazole by a thiazole ring.

Results and Discussion

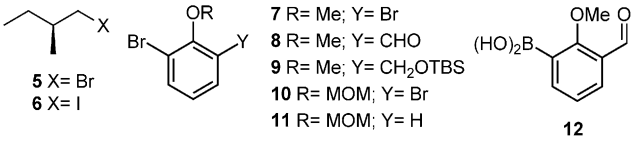
Syntheses of western fragments: Our principal retrosynthesis plan towards new derivatives of noricumazole A is summarized in Scheme 1. It is based on the key fusion of vinyl iodides **3** and aldehydes **4** by a nucleophilic vinylation (Scheme 1). The western fragment has a typical polyketide structure and could be synthesized by a classical aldol approach employing a Masamune *anti*-aldol[3] and a Nagao aldol[4] reaction for setting up the stereotriade. The syntheses of the western fragments were planned to start from a simple phenol precursor and, depending whether a substituent is present at C4, two to three successive organometallic transformations, which included a sp^3–sp^2 cross coupling reaction for R is unlike H.

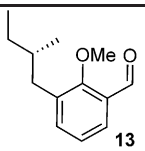
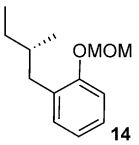


Scheme 1. General retrosynthesis plan.

For the key sp^3–sp^2 coupling reaction that introduces the aliphatic side chain various strategies were pursued, that rely on copper, palladium or iron catalysis either by using organometallics based on magnesium, zinc or boronates (Table 1). As aryl moieties we chose bromides **7** to **11** as well as boronic acid **12** of which aryl bromides **7–9** completely failed to serve as organomagnesium or organozinc intermediates in cross coupling reactions with alkyl halides **5** or **6**, respectively.[5]

Table 1. Selected examples of sp^2–sp^3 cross coupling reactions.



	Arene	Conditions	Product	Yield [%][a]
1	**12**	**5**, $Pd(OAc)_2$, KO*t*Bu, tBu_2MeP, amyl alcohol, room temperature	 **13**	24[b]
2	**11**	i. Mg, THF, Δ, then ii. PEPPSI-IPr™,[c] $ZnCl_2$, LiBr, **5**, DMI, THF, room temperature, 3 h	 **14**	n.p.[d,e]
3	11	Mg, TMEDA, THF, $FeCl_3$, **5** (1.2 equiv), 0 °C, 16 h, room temperature	**14**	33
4	**11**	Mg, TMEDA, THF, $FeCl_3$, **5** or **6** (1.2 equiv), 16 h	**14**	25 (for **5**) n.p. (for **6**) 50 (for **5**)[f]
5	**11**	i. Mg, THF, Δ, 1 h then ii. $Fe(acac)_3$, THF, TMEDA, HMTA, **6** (1 equiv), 0 °C, 1.5 h	**14**	98[g]

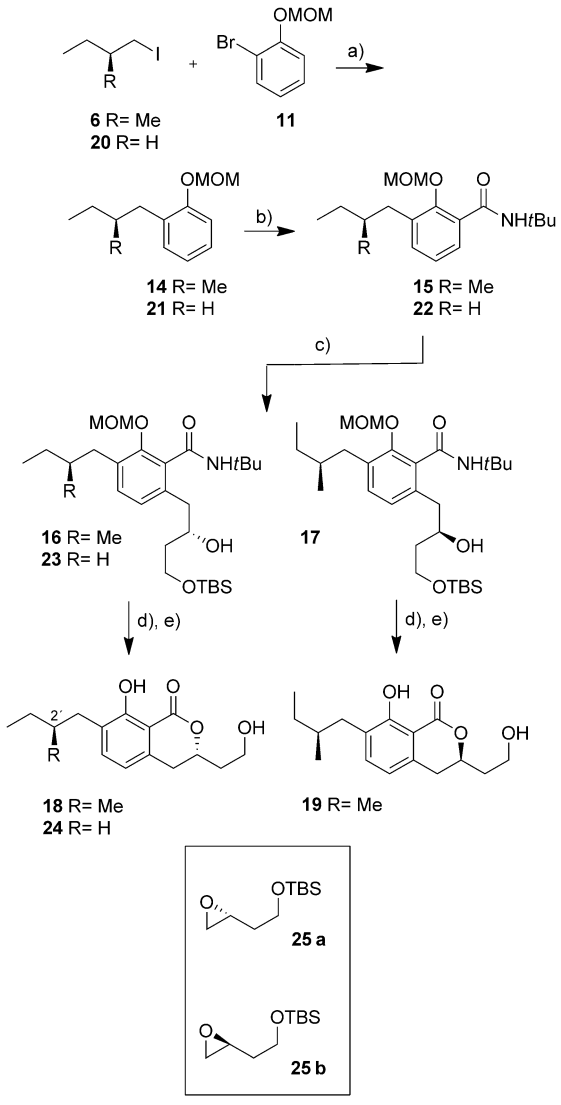
[a] Yields are based on isolated products; [b] crude yield; [c] for PEPPSI-IPr™ refer to ref. [7]; [d] n.p. = no product; [e] only the protodebrominated starting material was isolated; [f] distilled bromide **5** was employed; [g] freshly distilled iodide **6** and bromide **11** were employed; (DMI = 1,3-dimethyl-2-Imidazolidinone; TMEDA = tetramethylethylene diamine; acac = acetylacetonate; HMTA = hexamethylphosphortriamide).

The reversed mode of coupling, that is, transformation of the alkyl halides into the corresponding organometallic species and coupling with aryl bromides **7–9** was also not successful. Reactions were typically probed in the presence of a catalytic amount of an iron source and TMEDA or HMTA,[6] or in the presence of a palladium catalyst (e.g., PEPPSI-IPr™) following Organ's[7] or Fu's[8] procedures.

The first successful cross coupling reaction was achieved between boronic acid **12** and alkyl halide **6** following Fu's protocol (Table 1, entry 1);[8] however, the yield of 24% for **13** was far from satisfactory. Only, when the phenol protection was changed from the methyl to the MOM group the process could be improved. Employing the conditions described by von Wangelin and co-workers, aryl bromide **11** was coupled in the presence of iron (entry 3) instead of palladium (entry 2) to furnish arene **14** in 33% yield.[9] Finally, the iron-catalyzed version[7,9,10] of the Kumada reaction[11] gave the coupling product **14** in excellent yield (Table 1,

entry 5). The key to success was that besides TMEDA also freshly distilled alkyl halide and aryl bromide were employed.

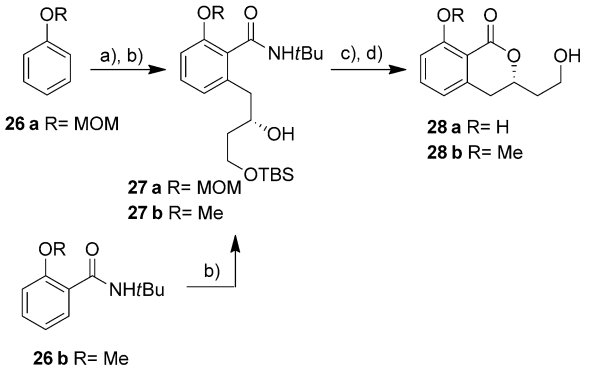
Next, the second *ortho*-position in **14** was functionalized by first deprotonating with *n*BuLi followed by treatment with *tert*-butyl isocyanate (Scheme 2). Amide **15** was obtained and then utilized to direct a second deprotonation and the subsequent *ortho* lithiated species was then independently treated with both enantiomeric oxiranes **25a**,**b**[12,13] to ring opening products **16** and **17**, respectively. These alcohols could only be transformed into the δ-lactones after removal of the MOM as well as the silyl protecting groups. Then, cyclization was achieved under acidic conditions in refluxing toluene to afford both diastereomeric fragments **18** and **19**.



Scheme 2. Preparation of western fragments **18** and **19** as well as 2′-nor-derivative **24**. Reagents and conditions: a) i. (*S*)-**6**, Mg, THF, Δ, 1 h; ii. **11**, HMTA, TMEDA, $Fe(acac)_3$, 0 °C, 1.5 h (**14**, 98 % and **21**, 76 % from **20**); b) i. *n*BuLi, TMEDA, Et_2O, −30 °C, 2 h, then 3 h at −5 °C; ii. *t*BuNCO, room temperature, 14 h, (94 % for **15** and 67 % for **22**); c) *n*BuLi, TMEDA, Et_2O, −78 °C to −40 °C, 2 h, then addition of (*R*)-**25a**, −78 °C to −40 °C, 14 h, [73 % (99 % b.o.r.s.m.) for **16** and 57 % (95 % b.o.r.s.m.) for **23**], alternatively, addition of (*S*)-**25b**, −78 °C to −40 °C, 14 h, (81 % for **17**); d) HCl_{conc}, EtOH, 50 °C, 2.5 h, (89 % from **16**, 93 % from **23** and 94 % from **17**); e) *p*TsOH, toluene, Δ, 30 min, (89 % for **18**, 77 % for **24** and 95 % for **19**); (TBS = *tert*-butyldimethylsilyl; MOM = methoxymethyl).

In a similar manner, we prepared the simplified western fragment **24** with an *n*-butyl side chain by following the synthesis protocols described for western fragments **18** and **19** (Scheme 2). The cross coupling reaction of butyl iodide **20** with aryl bromide **11** gave butylarene **21** in good yield.

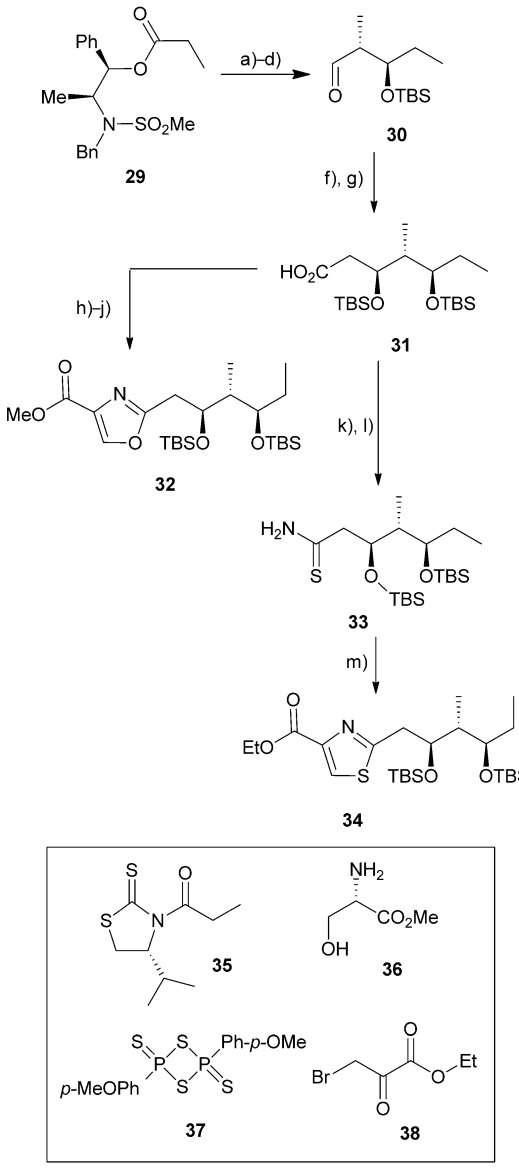
Additionally, also the western fragment that is not alkylated at C4 was prepared starting from arylether **26** (Scheme 3). Following the synthesis sequence described in Scheme 2 the isochromanone derivative **28a** and the *O*-methylated analogue **28b** were straightforwardly prepared.



Scheme 3. Preparation of western fragment derivative **28a** and **28b**. Reagents and conditions: a) i. *n*BuLi, TMEDA, Et_2O, −30 °C, 2 h, then 3 h at −5 °C; ii. *t*BuNCO, room temperature, 14 h, 89 %; b) *n*BuLi, TMEDA, Et_2O, −78 °C to −40 °C, 2 h, then addition of (*R*)-**25a**, −78 °C to −40 °C, 14 h [44 % (99 % b.o.r.s.m.) for **27a** and 76 % (99 % b.o.r.s.m.) for **27b**]; c) HCl_{conc}, EtOH, 50 °C, 50 min (70 % from **27a** and 99 % from **27b**); d) *p*TsOH, toluene, Δ, 45 min (98 % for **28a** and 92 % for **28b**).

Syntheses of eastern fragments: Based on the published synthesis,[1] we prepared one new eastern fragment that only differs in the heterocylic moiety compared to noricumazole A (**1a**). The natural oxazole ring was exchanged by thiazole moiety. The synthesis commenced with a Masamune *anti*-aldol reaction[2] between propionate **29** and propanal (Scheme 4). Aldehyde **30** was obtained after *O*-silylation, DIBAL-mediated reduction and Dess–Martin oxidation (Scheme 4).

The Nagao aldol reaction[3] served to establish the third stereogenic center by using thioxothiazolidine **35**. Second, *O*-silylation and hydrolytic removal of the chiral auxiliary gave the carboxylic acid **31**, which was coupled with serine methylester **36** in the presence of the condensation cocktail of HOBt and TBTU. In the presence of DAST[14] ring closure to the oxazolidine ring was initiated, which upon oxidation by using Williams' procedure led to the corresponding oxazole **32** in good overall yield.[15] In contrast, thiazole **34** was prepared via thioamide **33**, which was accessed from the



Scheme 4. Synthesis of the eastern thiazole fragment **33**. Reagents and conditions: a) *c*-HexBOTf, NEt_3, propanal, CH_2Cl_2, −78 °C, 2 h, 91 %, d.r. > 20:1; b) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C to room temperature, 50 min, quant.; c) DIBAL-H, CH_2Cl_2, −78 °C, 30 min, 91 %; d) Dess–Martin periodinane, CH_2Cl_2, room temperature, 1 h; e) **35**, $TiCl_4$, iPr_2EtN, −40 °C to −78 °C, 1.5 h; f) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 30 min, 91 % over three steps; g) 1 M LiOH, 30 % H_2O_2, THF/H_2O 4:1, 0 °C to room temperature, 12 h, 99 %; h) **36**, HOBt, TBTU, iPr_2EtN, CH_2Cl_2, room temperature, 12 h, 97 %; i) DAST, CH_2Cl_2, −78 °C, 3 h, 78 % (88 % b.o.r.s.m.); j) DBU, $BrCCl_3$, CH_2Cl_2, 0 °C to room temperature, 6 h, 85 %; k) i. $(COCl)_2$, DMF, Et_2O, room temperature, 1 h; ii. NH_4OH, MeOH, 0 °C, 1 h, 99 %; l) **37**, THF, room temperature, 4 h, 65 %; m) i. **38**, acetone, −10 °C, 2 h; ii. pyridine, $(CF_3CO)_2O$, CH_2Cl_2, −30 °C to room temperature over 4 h, 14 h, 88 %; (DAST = diethylaminosulfur trifluoride; DBU = 1,8-diazabicyclo[5.4.0]undec-7-en; DMF = *N*,*N*-dimethylformamid; DIBAL-H = diisobutylaluminium hydride; Hex = *n*-hexyl; HOBt = hydroxybenzotriazole; TBSOTf = *tert*-butyldimethylsilyl triflate; TBTU = *O*-benzotriazol-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate).

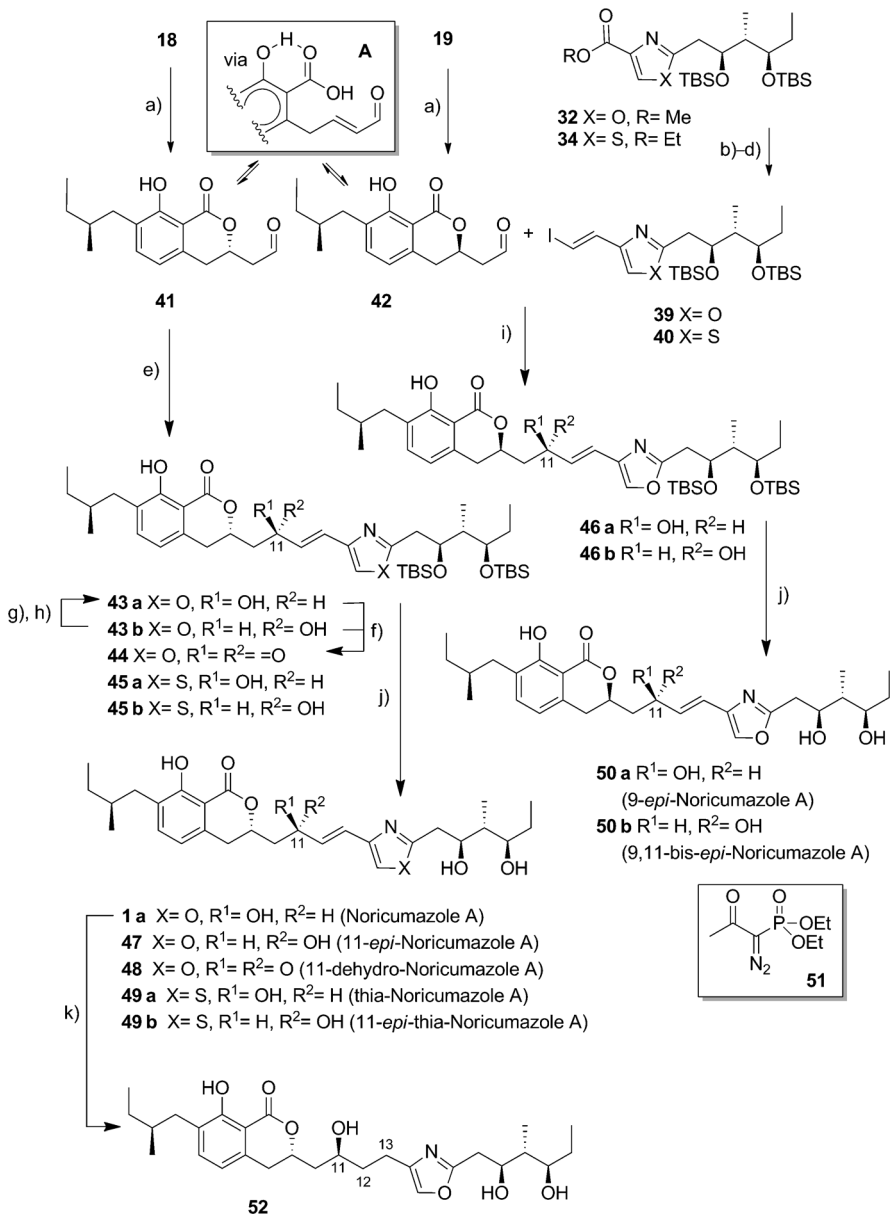
corresponding amide by thionation with Lawesson's reagent **37**.[16] Next, the Hantzsch thiazole synthesis by using bromopyruvate **38** gave the desired eastern fragment **34** in very good yield.[17]

With methyl ester **32** and ethyl ester **34** in hand, further elaboration to the corresponding vinyl iodides **39** and **40** (resembling **3**; Scheme 1), respectively, was pursued. This was achieved by first a reduction step yielding the corresponding aldehyde (Scheme 5) followed by Seyferth–Gilbert homologation[18] by using the Ohira–Bestmann reagent **51**.[19]

The resulting alkyne was subjected to a *syn*-hydro-zirconation[20] followed by iodination, which furnished vinyl iodides **39** and **40**, respectively. Noteworthy, we also tested the Takai olefination[21] of the intermediate aldehyde because this transformation would yield the desired vinyl iodides **39** and **40** in one step. However, we discovered that it was impossible to quantitatively isolate the vinyl iodide in the presence of excess iodoform.

The key step, the vinylation of aldehyde **41** with metalated vinyl iodide **39**, required substantial optimization. First of all, oxidation of the primary hydroxyl groups in **18** and its epimer **19** posed a problem because the β-center underwent facile epimerization for which we propose a β-elimination/oxo-Michael addition mechanism; this might be accelerated by hydrogen bonding between the phenol moiety and the lacton carbonyl group (see substructure **A**, Scheme 5). Epimerization could be suppressed when oxidation was carried out under strictly dry and acid-free conditions and the aldehyde was immediately employed in the next step.

Subsequently, vinylation was achieved after lithiation of vinyl iodide **39** and transmetalation to the corresponding organozinc species. Nucleophilic addition to aldehyde **41**[22] furnished the desired product **43a** and its 11-epimer **43b** as a 1:1 mixture.[1] Asymmetric coupling variants, like the use of chiral ligands[23] known to be applicable in the zinc-mediated vinylation or the chromium(II)-mediated Nozaki–Kishi reaction,[24] failed. Alternatively, we pursued the diastereoselective alkynylation of aldehyde **41** with lithiated alkyne collected en route to vinyl iodide **39** in the presence of various chiral ligands,[25] such as (−)-*N*-methylephedrine and (*R*)-(+)-1,1′-bi-2-naphthol. All these attempts were not successful. Thus, after chromatographic separation of both diastereomers the undesired 11-*epi*-allyl alcohol **43b** was directly epimerized to **43a** utilizing the Mitsunobu reaction[26] followed by ester hydrolysis. Following this route, we improved the yield for the desired coupling product **43a** to 68 %. The mixture of epimeric alcohols **43a** and **43b** could also be oxidized to the enone **44**. However, all attempts to achieve a facial-selective reduction by using (*S*)-2-methyl-CBS-oxazaborolidine, alpine–borane, Ipc_2BCl, L-Selectride or (*R*)-BINAL-H as reductants were not successful with respect to overall yield and selectivity.[27–29] Thus, completion of the synthesis was finally achieved by Lewis acid-mediated TBS-deprotection ($BF_3{\cdot}Et_2O$, CH_3CN, 0 °C, 12 h, 90 %) to furnish noricumazole A (**1a**) in 14 % overall yield over 15 steps (longest linear sequence; by including the Mitsunobu epimerization protocol the synthesis proceeded in 17 linear steps



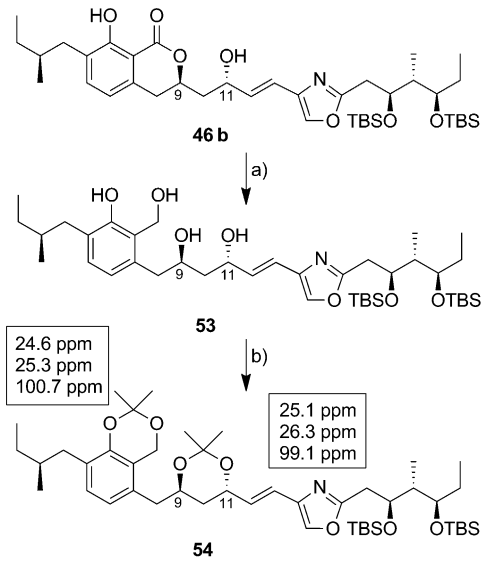
Scheme 5. Syntheses of noricumazole A (**1a**) and derivatives **47**–**50**. Reagents and conditions: a) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0 °C, 45 min, [85% for **41** and 44% (62% b.o.r.s.m.) for **42**]; b) DIBAL-H, CH_2Cl_2, −78 °C, 1 h, (quant. for both esters **32** and **34**); c) K_2CO_3, Ohira–Bestmann reagent **51**, MeOH, 0 °C to room temperature, (86% for oxazole and 96% for thiazole); d) Cp_2ZrHCl, THF, 0 °C, 1 h, then NIS, −78 °C, 45 min, (90% for **39** and 89% for **40**); e) **39**, *t*BuLi, Et_2O, −78 °C, 1 h, then Me_2Zn, −78 °C, 15 min, then **41**, Et_2O, −78 °C, 2.5 h, 74%, d.r. 1:1 (**43a**/**43b** from **41**) and 74%, d.r. 1:1 (**46a**/**46b** from **42**); f) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0 °C, 1 h, 65% for **44**; g) **43b**, PPh_3, *p*-nitrobenzoic acid, DEAD, THF, 0 °C to room temperature, 3 h, 90%; h) NaOH, H_2O, THF/MeOH 2:1, 0 °C, 12 h, 92%; i) **40**, *t*BuLi, Et_2O, −78 °C, 1 h, then Me_2Zn, −78 °C, 15 min, then **41**, Et_2O, −78 °C, 2.5 h, 74%, d.r. 1:1 (**45a**/**45b**); j) $BF_3{\cdot}Et_2O$, CH_3CN, 0 °C, 12 h (**1a**: 90%, **47**: 90%, **48**: 83%, **49a**: 51%, **49b**: 38%, **50a**: 83% and **50b**: 86%); k) Pd/C (10%), H_2 (1 bar), MeOH, room temperature, 2 h, 50%; (Cp = cyclopentadienyl; DEAD = diethyl azodicarboxylate; NIS = *N*-iodosuccinimide).

and 25% overall yield). In a similar manner we also obtained the 11-*epi* noricumazole A (**47**; 90%), 11-dehydro-noricumazole A (**48**; 83%), 9-*epi*-noricumazole A (**50a**; 83%) and 9,11-bis-*epi*-noricumazole A (**50b**; 86%) from the corresponding silyl-protected noricumazole derivatives **43b**, **44**, **46a** and **46b**, respectively.

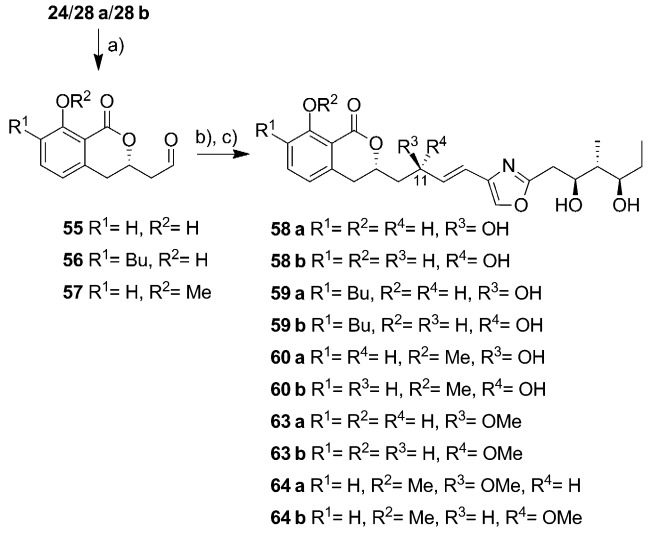
Likewise, the metallated vinyl iodide **40** that contains a thiazole instead of an oxazole heterocycle was coupled with aldehyde **41**. The two separable diastereomers **45a** and **45b** were also formed in a 1:1 ratio. Both diastereomeric thiazole derivatives were then deprotected to yield thia-noricumazole A (**49a**; 51%) and 11-*epi*-thia-noricumazole A (**49b**; 38%), respectively. Furthermore, we generated the 12,13-hydro-noricumazole A derivative **52** from noricumazole A (**1a**).

At this point it is necessary to briefly discuss how the stereochemistry at C11 with respect to C9 was elucidated, because the vinylation always provided two diastereomers. We addressed this issue in all cases as is exemplified for the 9,11-bis-*epi* derivative **46b** in Scheme 6.[30] First, the lactone moiety was reduced to yield the tetraol **53** followed by formation of bisacetonide **54**. According to Rychnovsky et al.[31] the ^{13}C δ values for the acetonide carbon atoms spanning C9 and C11 were diagnostic to unequivocally prove the 1,3-*anti* relationship. In case of the *n*-butyl side-chain-containing derivative **59a** and methyl protected phenol derivative **60a** the *anti* monoacetonides were formed.[30]

Having established a general route to new noricumazole derivatives we extended our efforts to a simplified *n*-butyl side chain at C3 as well as derivatives altogether without an alkyl side chain. These derivatives would provide information on the biological or pharmaceutical importance of this biosynthetically unusual aryl substituent. For this purpose alcohols **24**, **28a** and **28b** were oxidized to the corresponding aldehydes **55**–**57** and directly coupled with metallated vinyl iodide **39** to yield coupling products **58a**,**b**–**60a**,**b** as pair of diastereomers (1:1 ratio) after desilylation (Scheme 7).

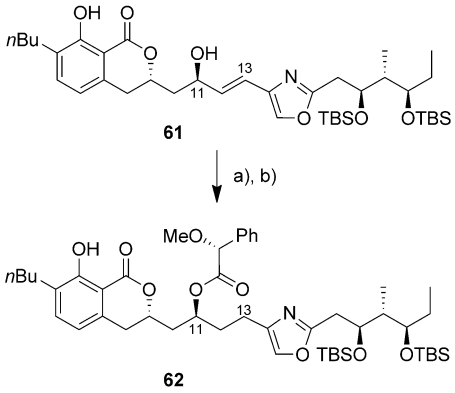


Scheme 6. Determination of the relative stereochemistry at C9 to C11 in **46b**. Reagents and conditions: a) **46b**, $LiBH_4$, THF, room temperature to 40 °C, 5.5 h; b) 2,2-dimethoxypropane, *p*TsOH, room temperature, 1.5 h, 37 % (for two steps; Ts = tosyl).



Scheme 7. Syntheses of noricumazole A derivatives **58**–**60** and byproducts **63** and **64**. Reagents and conditions: a) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0 °C, [49 % (60 % b.o.r.s.m.) for **56** (from **24**), 53 % for **55** (from **28a**) and 70 % for **57** (from **28b**)]; b) *t*BuLi, Et_2O, −78 °C, 1 h, then Me_2Zn, −78 °C, 15 min, then **39**, Et_2O, −78 °C, 3 h, 81 %, d.r. 1:1 [**58a**,**b** from **55**; 64 %, d.r. 1:1 (**59a**,**b** from **56**) and 31 %, d.r. 1:1 (**60a**,**b** from **58**)]; c) $BF_3{\cdot}Et_2O$, CH_3CN, 0 °C, 12 h, 72 % for **58a**, 40 % for **58b**, 81 % for **59a**, 90 % for **59b**, 53 % for **60a**, 29 % for **60b**.

The new derivatives lacking the stereogenic center at C2′, posed a challenge to us with respect to the determination of the degree of racemization at C9. For these derivatives that contain a methyl branch at C2' (e.g., **1a** and **47**) epimerization at C9 leads to two diastereomers that can be distinguished NMR spectroscopically by analysis of the chemical shift difference at H1' ($\Delta\delta = 0.02$ ppm).[1] This difference is diagnostic when epimerization at C9 has taken place during or after oxidation. However, for determining the stereogenic integrity at C9 of butyl-substituted derivatives, we pursued a strategy that is exemplified for derivative **61** (Scheme 8).



Scheme 8. Determination of the diastereomeric purity of **61**. Reagents and conditions: a) Pd/C (10 %), H_2 (1 bar), MeOH, room temperature, 1 h, 93 %; b) (*R*)-(−)-α-methoxyphenylacetic acid, EDC·HCl, DMAP, CH_2Cl_2, 40 °C, 40 min, 78 %; (DMAP = 4-dimethylaminopyridine; EDC = 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide).

In order to prevent epimerization at C11,[1] we first reduced the double bond at C12=C13. The saturated derivative was transformed into the mandelate derivative **62** for proving the diastereomeric purity of **61** by NMR spectroscopy.[32] Choosing the mandelic ester turned out to be advantageous compared to the Mosher ester[33,34] because the preparation of the latter derivative was always accompanied with the formation of the bisacylated products as well as with partial epimerization. When the stereochemical purity of derivatives **58a**,**b** and **60a**,**b**, that are devoid of the alkyl side chain, were studied in this way, unexpectedly the formation of two diastereomers was detected.[30] Again, we assume that epimerization took place at C9 this time during derivatization with *O*-methyl mandelic acid.[35] However, at this point we cannot fully exclude that epimerization at C9 takes place during formation of aldehydes **55** and **57**.

Thus, the alkyl side chain at C4 in noricumazole A (**1a**) and 2′-nor noricumazole A (**59a/b**) appears to have a stabilizing effect on the stereochemical integrity at the β-position of aldehydes **55** to **57** as well as the corresponding coupling products. Surprisingly, after HPLC purification of the reaction mixture that resulted from desilylation of **58a**, **58b**, **60a** and **60b** devoid of the aliphatic side, the 11-*O*-methylated byproducts **63a/b** and **64a/b** were isolated in small amounts (**63a**: 8 %; **63b**: 16 %; **64a**, 9 %; **64b**: 7 %; Scheme 7).[36] It can be assumed that the HPLC solvent MeOH served as methylating agent.

Biological evaluation: Recently, we disclosed a new dual reporter gene assay of the complete HCV life cycle that allows to identify small molecules that interfere with different steps of the HCV replication cycle.[2] The assay is able to discriminate between antiviral activity and cytotoxicity

(towards the host cell) and at the same time distinguishes between inhibitory influence on viral RNA translation and replication, and other steps of the viral life cycle. The assay is based on human hepatocarcinoma cells[37] and determines the efficiency of virus propagation in the presence or absence of compounds to be tested.

We specifically focused our attention to this bioassay since noricumazole A (**1a**) was identified to show antiviral activity against this important human pathogen in a high throughput screening.[2,38] Notably, thia-noricumazole A (**49a**) revealed strong anti-HCV activity with 50% inhibitory concentration (IC_{50}) of 16 nM (Figure 2). Importantly, it showed only moderate cytotoxicity with a 50% cytotoxic concentration (CC_{50}) of 350 nM, and therefore displays a good therapeutic index (i.e., $[CC_{50}]/[IC_{50}]$). Although the parent natural product, noricumazole A, shows enhanced anti-HCV activity compared to the thia-analogue **49a**, its therapeutic index is much worse due to pronounced cytotoxicity. In both cases, epimerization at C11 (e.g., **47** and **49b**) or oxidation at C11 (**48**) led to weak HCV inhibitors. Epimerization at C9 has a small deactivating effect although still good activity was retained for 9-*epi*-noricumazole A (**50a**). The trend noted for 11-epimerization holds in the case of the 9-*epi* compound, **50b**, too. When the olefinic double bond at C12=C13 is saturated (**52**) anti-HCV activity remains in the same range as determined for noricumazole A. When altering the isobutyl side chain to the *n*-butyl group (**59a**,**b**) still anti-HCV inhibitory activity is retained, however, it is reduced by a factor of approximately 12 for **59b** and by about 2 for **59a** compared to noricumazole (**1a**). Finally, noricumazole A derivatives (**58**, **60**, **63** and **64**) devoid of the alkyl side chain only showed low activity with IC_{50} values 70-fold or more of that determined for noricumazole (**1a**).

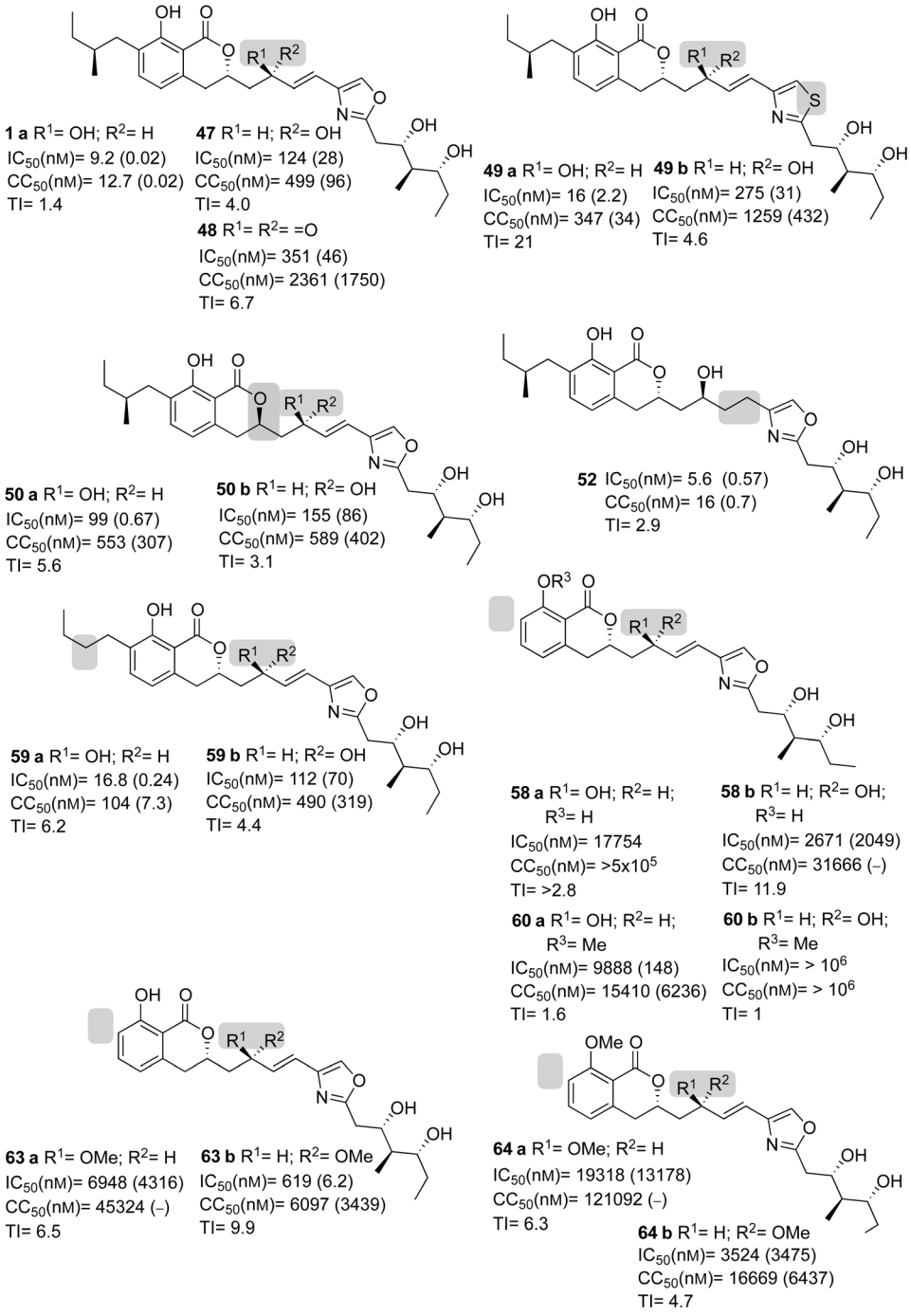


Figure 2. Inhibitory concentration (IC_{50}) on HCV life cycle, cytotoxic concentration (CC_{50}) on host cell (human hepatocarcinoma cells) and therapeutic index (TI). Structural changes with reference to noricumazole A (**1a**) are marked in gray; values in parentheses refer to standard deviations.[a]

In summary, these findings highlight thia-noricumazole A (**49a**) to be a lead candidate for further development as an antiviral compound against HCV. It is highly potent with an already excellent therapeutic index. In future studies it will be of interest to prepare a small set of thia-noricumazole derivatives and find out whether **49a** impedes HCV replication by interfering with the HCV p7 ion channel.[39]

Conclusion

In conclusion, we report the total synthesis of a small library of noricumazole A that relied on a convergent strategy with two fragments of equal complexity. Diversity was established by inverting selected stereocenters, simplification of the aryl-bound alkyl chain and by exchanging the oxazole moiety by a thiazole ring. All new noricumazole A derivatives were evaluated as inhibitors of the hepatitis C virus (HCV) by using a dual reporter gene assay of the complete HCV life cycle. The thiazole analogue of noricumazole A was found to be a strong inhibitor of HCV replication in the lower nM range and a therapeutic index of greater than 10. Therefore, this noricumazole A derivative can be regarded as a lead structure for the development of an anti-HCV agent.

Experimental Section

Descriptions of all experimental procedures and analytical characterizations as well as copies of the NMR spectra of the new compounds can be found in the Supporting Information.

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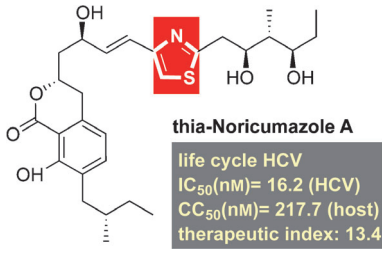
Sulfur matters! A library of 16 noricumazole A derivatives was prepared by total synthesis. Noricumazole A is a strong inhibitor of the hepatitis C virus (HCV) life cycle but also shows pronounced cytotoxicity. In contrast, the new thiazole analogue (see structure) may be regarded as a lead structure with a good therapeutic index (CC_{50}/IC_{50}) of greater than 10.



Natural Products

*J. Barbier, J. Wegner, S. Benson, J. Gentzsch, T. Pietschmann, A. Kirschning** ..... ▮▮▮▮–▮▮▮▮

Total Synthesis of a Noricumazole A Library and Evaluation of HCV Inhibition