Improved Convergent Synthesis of 5′-*epi*-Analogues of Muraymycin Nucleoside Antibiotics

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Received 8 June 2009

Dedicated to Professor Armin de Meijere on the occasion of his 70th birthday.

Abstract: Nucleoside antibiotics represent a promising class of natural products for the development of novel antibacterial agents, with particular respect to structurally simplified analogues maintaining biological activity. There are established truncated 5′-*epi*-derivatives of muraymycin nucleoside antibiotics with reported antibacterial properties, but the lengthy preparation of such compounds is a major hurdle in more detailed structure–activity relationship (SAR) studies. A concise, improved synthesis of truncated 5′-*epi*-muraymycins based on a previously reported approach using sulfur ylide chemistry is reported here. The highly convergent nature of this strategy will allow the efficient synthesis of novel muraymycin analogues for thorough SAR investigations.

Key words: antibiotics, medicinal chemistry, MraY inhibitors, natural products, nucleosides

Due to emerging resistances of bacteria towards established antibiotics,[1] there is an urgent need to develop novel antibacterial agents, ideally with new or yet unexploited modes of action.[2] One attractive target for new antibiotics might be the bacterial membrane protein translocase I (MraY), a key enzyme in the early stages of peptidoglycan biosynthesis.[3–5]

Nucleoside antibiotics represent an interesting class of natural products with often unusual nucleoside structures and display inhibitory potency towards MraY.[6] Muraymycin nucleoside antibiotics have been isolated from a *Streptomyces* sp. as a collection of 19 compounds [e.g., muraymycin A1 (**1**), Figure 1].[7] Following initial structure–activity relationship (SAR) studies using a semisynthetic approach,[8a] a series of synthetic truncated muraymycin analogues has been described.[8b] Some of these derivatives (e.g., compounds **2**–**6**, Figure 1) were reported to have remarkable activities against *Staphylococcus aureus* and were identified as MraY inhibitors.[8b] However, SAR investigations on such muraymycin analogues led to surprising results: firstly, the absolute configuration at the 5′-position was required to be *R* in order to obtain good activities, that is, only 5′-*epi*-analogues with respect to the 5′*S*-configured natural products were active. Secondly, the presence of some synthetic protecting groups [*tert*-butyl ester, *tert*-butyldimethylsilyl (TBDMS) groups] turned out to be a prerequisite for antibacterial potency. Only the *para*-methoxybenzyl (PMB) group at the uracil-N-3 could be omitted without decreasing the biological activity, which was demonstrated by the pronounced antibacterial activity of muraymycin analogue **6**.

The synthesis of 5′-*epi*-muraymycin analogues was reported either via aldol chemistry[8b] or via opening of an epoxide. This epoxide, in turn, was prepared stereoselectively from protected uridine 5′-aldehyde using a sulfur ylide.[9] The reported aldol reaction gave moderate yields

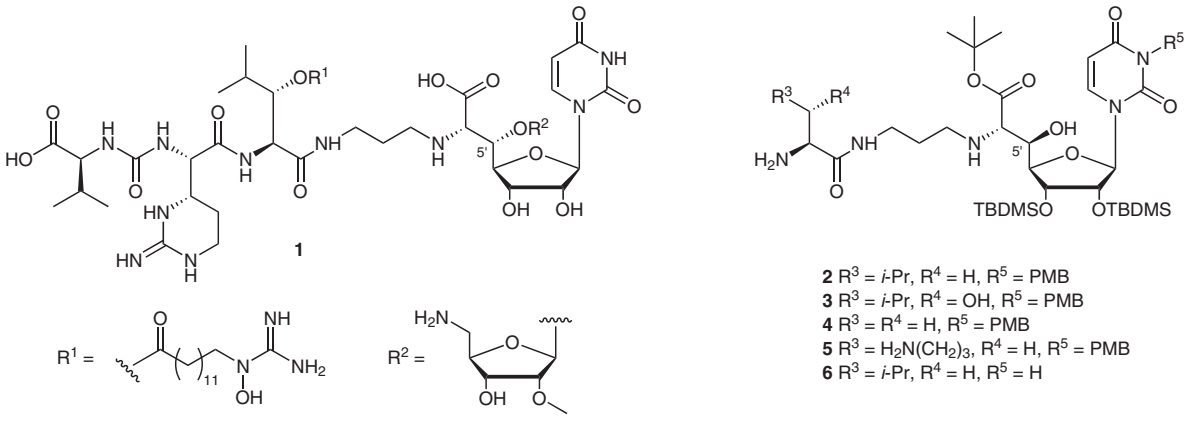

Figure 1 Naturally occurring nucleoside antibiotic muraymycin A1 (**1**) and synthetic truncated 5′-*epi*-muraymycin analogues **2**–**6** displaying antibacterial activity.[8b] Compound **2** has been chosen as target structure for this study.

SYNLETT 2009, No. 15, pp 2503–2507
Advanced online publication: 27.08.2009
DOI: 10.1055/s-0029-1217742; Art ID: G18709ST
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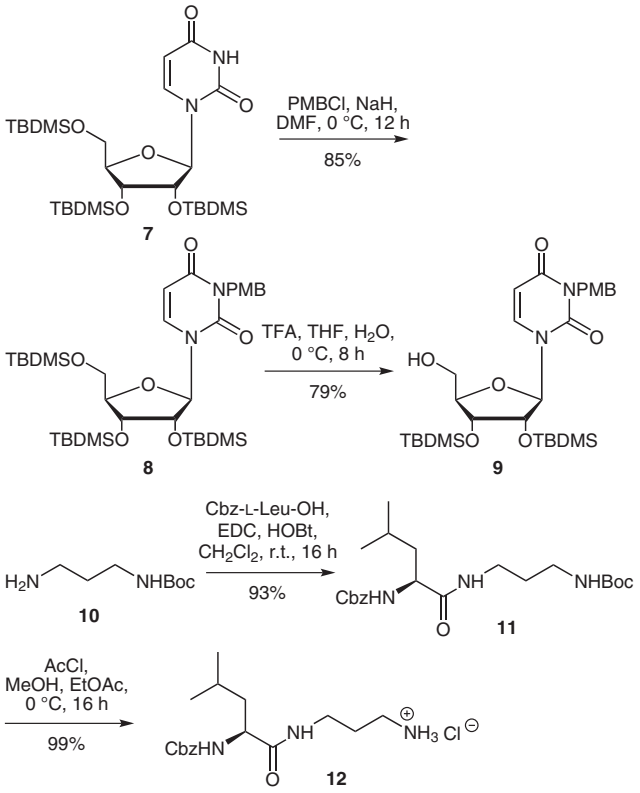
and stereoselectivities in our hands, requiring tedious chromatographic separation of the obtained diastereomeric mixtures. Hence, the objectives of this study were to synthesise arbitrarily chosen target compound **2** following the sulfur ylide pathway[9] and to possibly shorten and simplify this synthetic route to allow an efficient, convergent, and modular access of 5′-*epi*-muraymycins. Though the methodology of the sulfur ylide approach was established, it had not been applied to the synthesis of **2** before. Here we describe the synthesis of this compound both via previously reported transformations[9] and employing a significantly shortened and improved procedure.

Initially, two synthetic building blocks were required (Scheme 1). In order to avoid an additional step for selective 5′-O-trityl protection of the uridine derivative, trisilylated uridine **7**[10] (which can be easily prepared from uridine with TBDMS chloride and imidazole in pyridine as solvent in quantitative yield) was converted into PMB-protected derivative **8** in 85% yield. Selective acidic 5′-O-desilylation[11] then gave the protected uridine building block **9**[12] (79% yield). For the construction of the peptidic muraymycin side chain, commercially available mono-Boc-protected 1,3-diaminopropane **10** was used in a standard peptide-coupling reaction with *N*-Cbz-protected L-leucine to afford **11** in 93% yield, and acidic Boc deprotection then furnished the amino-acylated side-chain building block **12** (99% yield).

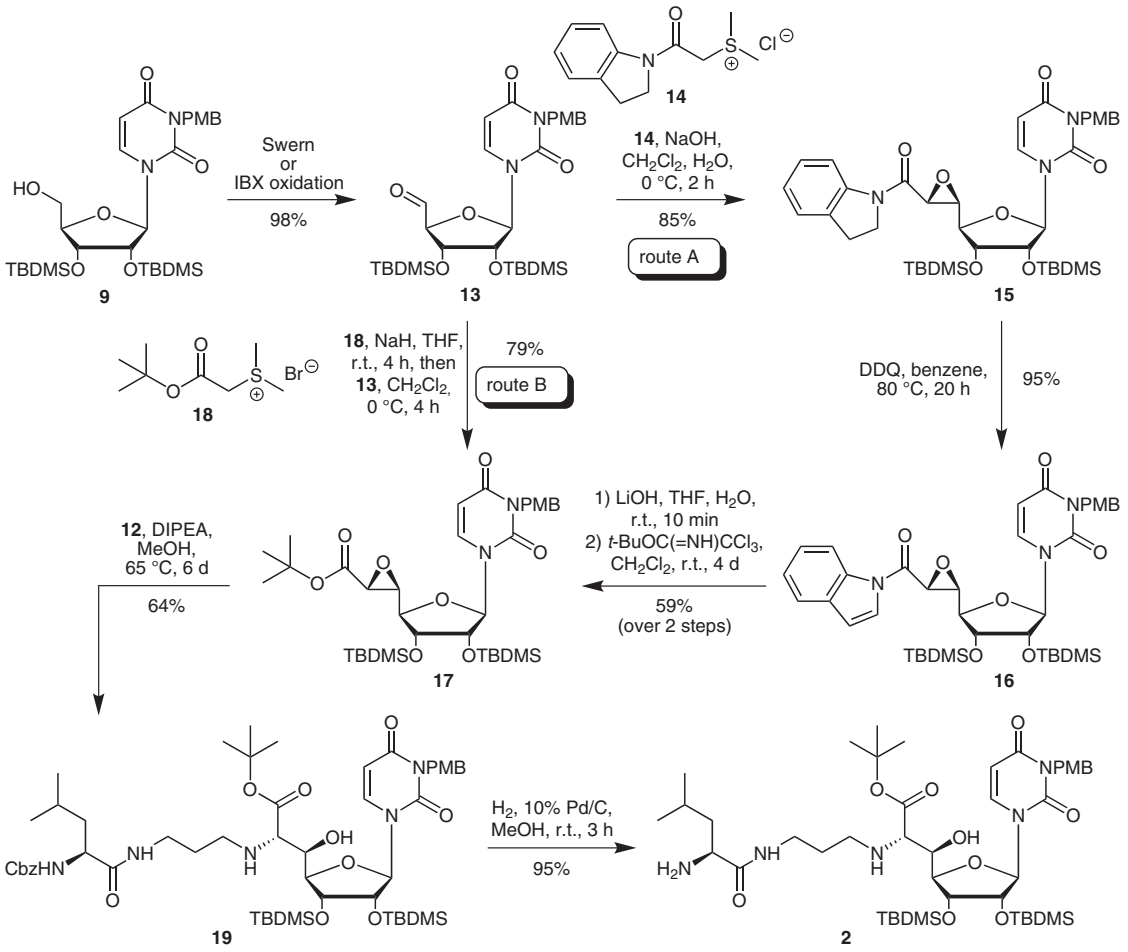
For the application of the sulfur ylide route, protected uridine 5′-aldehyde **13**[8b,12] was required, which could be readily obtained either by Swern or by IBX oxidation of alcohol **9** in nearly quantitative yield (Scheme 2). However, this compound was found to be sensitive and instable, and consequently, it had to be prepared freshly prior to sulfur ylide reactions and was directly used without further purification. Employing the established strategy (route A, Scheme 2), aldehyde **13** was reacted with a sulfur ylide generated in situ from sulfonium salt **14**[13] to give the epoxide **15** with excellent diastereoselectivity (dr > 97:3 as judged by NMR) and good yield (85%). The anticipated *trans* stereochemistry of the epoxide moiety in **15** could be unambiguously assigned based on ^{1}H NMR coupling constants, and the absolute configuration of the two newly formed stereocentres had been determined before.[9] However, further conversion of **15** into a suitable building block for completion of the synthesis of **2** was lengthy. After chemoselective oxidation of indoline amide **15** to the indole amide **16** with DDQ (95% yield), saponification of the amide moiety furnished an epoxy carboxylic acid. Due to severe problems to purify this compound, the crude product obtained from the saponification reaction was directly converted into the *tert*-butyl ester **17**, which could be isolated in 59% yield over two steps from **16** after chromatography. In summary, protected uridine derivative **9** could be converted into key intermediate **17** in an overall yield of 47% over five steps via this route.

This lengthy five-step transformation of **9** into the desired epoxide **17** was expected to limit the convergence and modularity of the synthetic strategy. Using a novel approach, the synthetic sequence could be significantly shortened as the epoxide-forming reaction was conducted with a sulfur ylide generated from sulfonium salt **18** (route B, Scheme 2). This readily prepared *tert*-butyl ester derivative,[14] which had found application in sulfur ylide chemistry before,[15] provided efficient direct access to key intermediate **17** in 79% yield from **13** (78% yield over 2 steps from **9**, including only one chromatographic purification).[16,17] The excellent diastereoselectivity of the sulfur ylide utilising step (vide supra) was retained, and the isolated epoxide was spectroscopically (NMR) and chromatographically (HPLC coinjection) identical with the material obtained via the reported route described above (route A, Scheme 2).

Finally, assembly of the 5′-*epi*-muraymycin structure was accomplished by stereospecific regioselective opening of epoxide **17** with amine **12** leading to **19** (64% yield, Scheme 2). In the previous report on the sulfur ylide strategy,[9] epoxide opening was performed with derivatives of 1,3-diaminoalkanes in most cases. With respect to a high convergence of the synthetic route, however, it was desired to avoid additional steps and the late-stage introduction of the amino acid moiety. Monoaminoacyl 1,3-diaminopropane **12** was therefore used as a nucleophile for the reaction with epoxide **17**. The diastereo- and regioselectivity of this reaction was unambigously proven by NMR-spectroscopic investigation of isolated product **19**. Target compound **2** was then obtained after hydrogenolytic Cbz-deprotection of **19** in 95% yield for the final step



Scheme 1

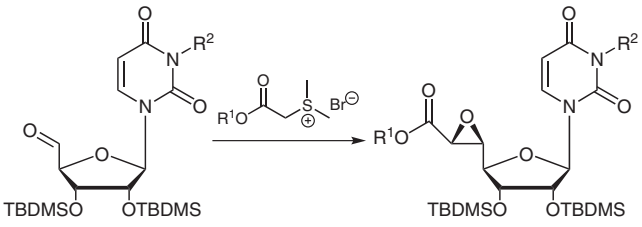


Scheme 2

and in an overall yield of 32% over seven linear steps from uridine.

The use of different ester-derived sulfonium salts and nucleoside derivatives in the sulfur ylide reaction would provide rapid access to 5′-*epi*-muraymycin analogues with different ester moieties and nucleoside structures, thus allowing more detailed SAR studies. The scope of the novel 'direct' sulfur ylide approach was therefore further investigated (Table 1). A series of different sulfonium salts[14] was used to generate sulfur ylides to be reacted with aldehyde **13**. Yields varied and were found to be moderate (e.g., 30% for R = Me, entry 2 in Table 1) to good (e.g., 80% for R = Et, entry 3). 5′-*epi*-Muraymycin analogues without nucleobase protecting groups such as **6** (Figure 1) were reported to be bioactive as well,[8b] and deprotection of N-3-PMB-protected muraymycin derivatives with CAN led to complex product mixtures in our hands. We therefore also investigated the conversion of aldehyde **20**[9] not bearing a nucleobase protecting group with the sulfur ylide derived from sulfonium salt **18** and obtained the corresponding epoxy ester product in good yield (60%, entry 6, Table 1). As described before for epoxide **17**, this reaction product was found to be identical to the one synthesised via the established route.[18] All sulfur ylide reactions depicted in Table 1 led to exclusive formation of just one

Table 1 Reaction of Uridine Aldehydes **13** and **20** with Sulfur Ylides Generated from Different Ester-Derived Sulfonium Salts



Entry	Aldehyde	R^1	Yield of epoxy ester product (%)[a]
1	**13**	*t*-Bu	79
2	**13**	Me	30
3	**13**	Et	80
4	**13**	*n*-Pr	37
5	**13**	Bn	53
6	**20**	*t*-Bu	60

[a] Isolated yields in % with preparation of the aldehyde directly prior to the sulfur ylide reaction.

trans-epoxide diastereomer. The absolute configuration of the two newly formed stereocentres, which had been indirectly determined for the products of Table 1, entries 1 and 6 (vide supra), was proposed to be identical throughout.[9]

In conclusion, we report an improved synthesis of 5′-*epi*-analogues of muraymycin nucleoside antibiotics using a novel 'direct' sulfur ylide approach. This route bypasses several steps of the previously reported methodology for 5′-*epi*-muraymycin synthesis while retaining its excellent stereoselectivity, therefore giving rise to a highly convergent strategy. The longest linear sequence from uridine to a target structure is comprised of seven steps only. The apparent broad scope of the newly established 'direct' sulfur ylide reaction will allow the rapid modular synthesis of novel muraymycin analogues, which can then be screened for MraY inhibitor activity.

Acknowledgment

We thank the Deutsche Forschungsgemeinschaft (DFG, SFB 803 'Functionality controlled by organization in and between membranes') and the Fonds der Chemischen Industrie (FCI) for financial support. Donation of laboratory equipment by the BASF SE is gratefully acknowledged.

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(14) **Synthesis of Ester-Derived Sulfonium Salts**
A solution of the respective alkyl bromoacetate in degassed dimethylsulfide (*tert*-butyl, *n*-propyl and benzyl esters) or in acetone–dimethylsulfide (methyl ester) was stirred at r.t. for 2 d. The precipitated product was subsequently filtered off, washed with *n*-hexane or PE, and dried in vacuo. With exception of the *tert*-butyl ester derivative, the obtained sulfonium salts still contained significant amounts of trimethylsulfonium bromide, but could be used for sulfur ylide generation in crude form. The ethyl ester derivative was commercially available.
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(16) **Synthesis of Epoxy Ester 17 via 'Direct' Sulfur Ylide Reaction**
A solution of sulfonium salt **18** (430 mg, 1.67 mmol) in dry THF (10 mL) was stirred over 4 Å MS at r.t. for 2 h to remove any traces of H_2O from the hygroscopic sulfonium salt. Sodium hydride (60% suspension in mineral oil, 68 mg, 1.7 mmol) was then added at 0 °C, and the mixture was stirred at r.t. for 4 h. After filtration and evaporation of the solvent under reduced pressure, the obtained sulfur ylide was dissolved in dry CH_2Cl_2 (2 mL). This solution of the sulfur ylide was added in aliquots (0.5 mL each) at 0 °C over a period of 4 h to a stirred solution of uridine aldehyde **13** (99 mg, 0.168 mmol, freshly prepared by IBX oxidation of protected uridine **9** in MeCN) in dry CH_2Cl_2 (2 mL). After addition of more CH_2Cl_2 (25 mL) and H_2O (25 mL), the aqueous layer was extracted with EtOAc (25 mL). The combined organics were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The resultant crude product was purified by column chromatography (PE–EtOAc, 9:1) to give **17** (93 mg, 79%) as a colourless solid; mp 71 °C. TLC: R_f = 0.50 (PE–EtOAc, 7:3). $[\alpha]_D^{20}$ +31.6 (*c* 1.3, $CHCl_3$). ^{1}H NMR (300 MHz, C_6D_6): δ = –0.06 (s, 3 H, $SiCH_3$), –0.01 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.87 [s, 9 H, $SiC(CH_3)_3$], 0.93 [s, 9 H, $SiC(CH_3)_3$], 1.33 [s, 9 H, $OC(CH_3)_3$], 3.24 (s, 3 H, OCH_3), 3.39 (dd, *J* = 1.3, 1.3 Hz, 1 H, 5′-H), 3.65 (d, *J* = 1.3 Hz, 1 H, 6′-H), 3.97 (dd, *J* = 4.3, 4.3 Hz, 1 H, 3′-H), 4.06 (dd, *J* = 4.3, 1.3 Hz, 1 H, 4′-H), 4.10 (dd, *J* = 4.3, 4.3 Hz, 1 H, 2′-H), 5.04 (d, *J* = 13.3 Hz, 1 H, PMB-CH_2-H_a), 5.12 (d, *J* = 13.3 Hz, 1 H, PMB-CH_2-H_b), 5.46 (d, *J* = 8.1 Hz, 1 H, 5-H), 6.10 (d, *J* = 4.3 Hz, 1 H, 1′-H), 6.72 (d, *J* = 8.2 Hz, 2 H, PMB-3-H, PMB-5-H), 7.42 (d, *J* = 8.1 Hz, 1 H, 6-H), 7.67 (d, *J* = 8.2 Hz, 2 H, PMB-2-H, PMB-6-H). ^{13}C NMR (75 MHz, C_6D_6): δ = –5.1 ($SiCH_3$), –4.7 ($SiCH_3$), –4.5 ($SiCH_3$), –4.5 ($SiCH_3$), 18.2 [$SiC(CH_3)_3$], 25.9 [$SiC(CH_3)_3$], 26.0 [$SiC(CH_3)_3$], 27.8 [$OC(CH_3)_3$], 43.7 (PMB-CH_2), 51.2 (C-6′), 54.6 (OCH_3), 56.9 (C-5′), 73.7 (C-3′), 75.6 (C-2′), 78.9 (C-4′), 82.5 [$OC(CH_3)_3$], 89.2 (C-1′), 102.7 (C-5), 114.0 (PMB-C-3, PMB-C-5), 129.8 (PMB-C-1), 131.5 (PMB-C-2, PMB-C-6), 136.5 (C-6), 151.4 (C-2), 159.7 (PMB-C-4), 161.8 (C-4), 167.1 (ester C=O). MS (ESI$^+$): *m/z* = 727.4 [M

+ Na]$^+$. HRMS (ESI$^+$): *m/z* calcd. for $C_{35}H_{56}N_2O_9Si_2$: 705.3597 [M + H]$^+$; found: 705.3597 [M + H]$^+$. IR (KBr): ν = 2932, 1671, 1514, 1456, 1392, 1250, 1162, 839, 778 cm^{-1}. UV (MeCN): λ_{max} (lg ε) = 194 (4.69), 222 (4.13), 262 (3.96).

(17) When a procedure similar to the one used for the synthesis of **15** via route A (Scheme 2) with in situ generation of the sulfur ylide from **18** was applied, epoxy ester **17** could also be obtained, though in lower yield (60%).

(18) Aldehyde **20** was also converted into the respective epoxy ester product using transformations according to route A in Scheme 2 as reported previously.[9]

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