General syntheses of 1-alkyltoxoflavin and 8-alkylfervenulin derivatives of biological significance by the regioselective alkylation of reumycin derivatives and the rates of transalkylation from 1-alkyltoxoflavins into nucleophiles

Tomohisa Nagamatsu * and Hirofumi Yamasaki

Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700-8530, Japan. E-mail: nagamatsu@pheasant.pharm.okayama-u.ac.jp

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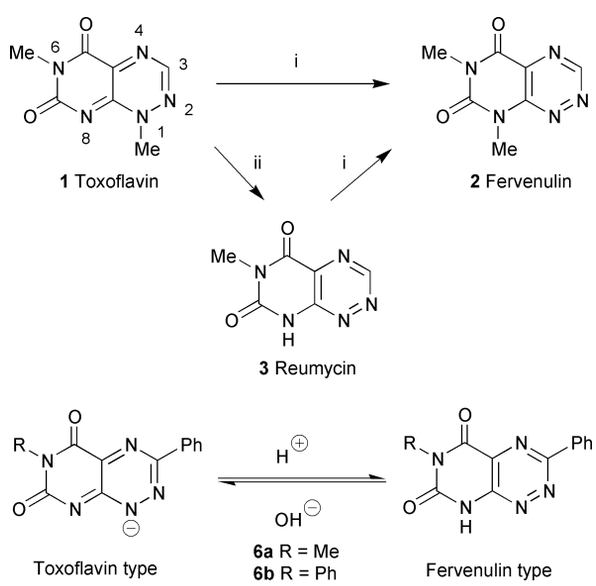
Regioselective alkylations of reumycin derivatives under alkaline conditions with a dialkyl sulfate or alkyl halide in 1,4-dioxane or DMF to provide 1-alkyltoxoflavin or 8-alkylfervenulin derivatives of biological significance, are described. Namely, the primary and secondary alkylations of reumycin derivatives with appropriate dialkyl sulfates or alkyl bromides under alkaline conditions in 1,4-dioxane gave predominantly 1-alkyltoxoflavin derivatives, while the same alkylations in DMF instead of 1,4-dioxane gave predominantly 8-alkylfervenulin derivatives. In the case of tertiary alkylation, the reumycin derivative with 2-bromo-2-methylpropane in both solvents under the same conditions yielded only the 1-alkyltoxoflavin derivative. Moreover, the rates of transalkylation from 1-alkyltoxoflavin derivatives into nucleophiles, *e.g.* DMF and *n*-butylamine, are also described. That is, the toxoflavin derivatives possessing a primary alkyl group at the 1-position were easily dealkylated from the 1-position by heating with DMF, whereupon reumycin (*i.e.*, 1-dealkyltoxoflavin, 8-dealkylfervenulin) derivatives were formed. In other words, transalkylation from the toxoflavin derivatives into DMF took place. However, the transalkylation of 1-alkyltoxoflavin derivatives possessing a secondary or tertiary alkyl group at the 1-position was not observed under such conditions. On the other hand, when heating 1-alkyltoxoflavin derivatives with *n*-butylamine in 1,4-dioxane, the transalkylations were more easily observed even in the case of 1-alkyltoxoflavin derivatives substituted by a tertiary alkyl group.

Introduction

Since the isolation and characterisation of the naturally occurring antibiotics of 7-azapteridines (pyrimido[5,4-*e*][1,2,4]-triazines), *e.g.* toxoflavin **1**,[1] fervenulin **2**[2] and reumycin **3**[3] isolated from *Pseudomonas cocovenenans*, *Streptomyces fervens* n. sp. and *Actinomyces*, respectively, the 7-azapteridines have been the subject of a great deal of synthetic study,[4] because of their marked biological activities[5,6] (Scheme 1). We have recently developed several convenient synthetic procedures for preparation of toxoflavin **1** and its 3- and/or 6-substituted derivatives,[7] and evaluated their potent anti-viral[6] and anti-tumour activities[8] and their ability as herbicides.[9] These findings prompted us to explore synthetic methods to prepare 1-substituted toxoflavin type derivatives and the structure–activity relationships for such activities. However, we encountered difficulties when attempting to prepare the derivatives possessing a substituent of some kind at the 1-position of the toxoflavin skeleton **1** by a method of nitrosative or nitrative cyclisation of the aldehyde hydrazones of 6-(1-alkylhydrazino)uracils.[6,7] Because it was not easy to get monosubstituted hydrazines except for methylhydrazine, the preparation of several 6-(1-alkylhydrazino)uracils as intermediates of the hydrazones was difficult. Moreover, we have previously reported that toxoflavin **1** and its 3-substituted derivatives readily undergo demethylation at the 1-position upon heating with some nucleophiles, *e.g.* DMF and dimethylacetamide, to give the corresponding 1-demethyltoxoflavin (*i.e.*, 8-demethylfervenulin, reumycin) derivatives, while the nucleophiles themselves were methylated by the methyl group eliminated, and during the reactions novel radical species were observed.[10] On the other hand, the methylation of reumycin and its 3-substituted derivatives under alkaline conditions with dimethyl sulfate or methyl iodide in DMF provided not toxoflavins but fervenulins.[11]



Scheme 1 *Reagents and conditions*: i, MeI, K_2CO_3, DMF, reflux; ii, DMF, reflux.

We have formerly investigated that the biological activities of toxoflavins were mostly stronger than those of fervenulins and reumycins.[6,8,9] Despite various biological activities being expected for the 1-substituted toxoflavin derivatives, no report

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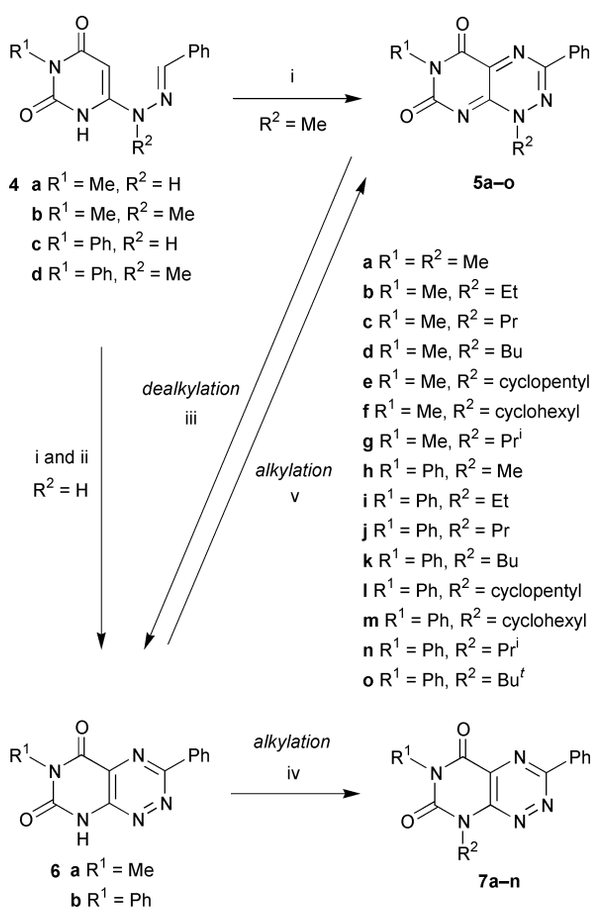
on the synthesis except for the methyl derivatives at the 1-position is available so far. In our recent communication[12] and a patent,[13] we reported a convenient and general methodology for the preparation of 1-alkyltoxoflavin derivatives by the regioselective alkylation of reumycin derivatives under alkaline conditions with a dialkyl sulfate or alkyl halide in 1,4-dioxane. Herein we report full details of the general syntheses of 1-alkyltoxoflavin and 8-alkylfervenulin derivatives by the regioselective alkylation of reumycin derivatives. Furthermore, we also report here the rates of transalkylation from 1-alkyltoxoflavins into nucleophiles, *e.g.* DMF and *n*-butylamine.

Results and discussion

As we reported previously, 3-phenyltoxoflavin **5a** was transformed into 3-phenylfervenulin **7a** *via* 3-phenylreumycin **6a** by heating with methyl iodide and potassium carbonate in DMF in usual alkylating conditions.[11] As a consequence of the demethylation at the 1-position of 3-phenyltoxoflavin **5a** due to DMF, the alkylation of the resulting 3-phenylreumycin **6a** by methyl iodide proceeded predominantly in the direction of the 8-position to afford 3-phenylfervenulin **7a**. In a similar manner as noted above, we also observed the transformation of 3,6-diphenyltoxoflavin analogue **5h** into 3,6-diphenylfervenulin analogue **7h** as described in the Experimental section (Scheme 2).



Scheme 2 *Reagents and conditions*: i, $NaNO_2$, AcOH, 5–10 °C; ii, Ac_2O, reflux; iii, DMF, 140 °C or $BuNH_2$, 1,4-dioxane, 100 °C; iv, $(R^2O)_2SO_2$, R^2I or R^2Br, K_2CO_3, DMF, 140 °C; v, $(R^2O)_2SO_2$ or R^2Br, K_2CO_3, 1,4-dioxane, 120 °C.

Liao *et al.*[14] suggested that an equilibrium between toxoflavin type and fervenulin type structures existed under different environmental conditions with respect to reumycin **3**. We also observed a similar equilibrium in comparison of the ultraviolet

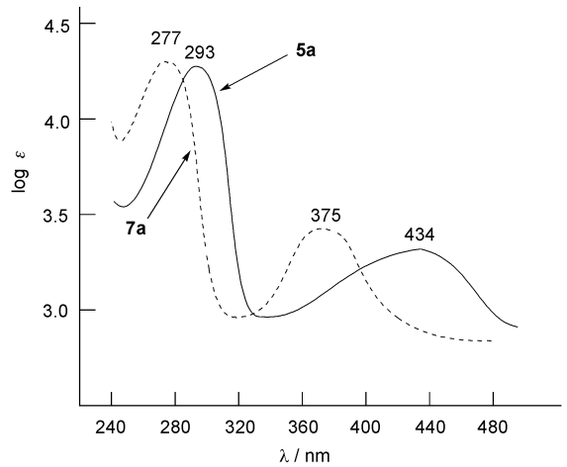


Fig. 1

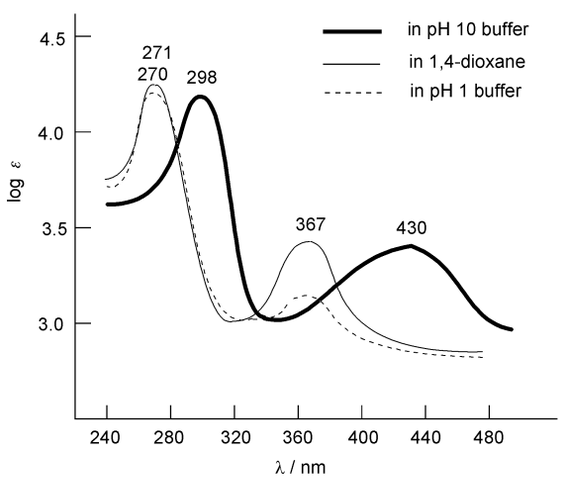


Fig. 2

absorption spectra of 3-phenylreumycins **6a**,**b** in different media as shown in Scheme 1 and the Experimental section. The UV spectrum of 3-phenyltoxoflavin **5a** in 1,4-dioxane showed two maxima at 293 and 434 nm, while that of 3-phenylfervenulin **7a** showed two maxima at 277 and 375 nm (Fig. 1). The spectra of 3-phenylreumycin **6a** in 1,4-dioxane (maxima at 271 and 367 nm) and in acidic medium (pH 1, maxima at 270 and 367 nm) resemble that of 3-phenylfervenulin **7a** in 1,4-dioxane (Fig. 2). On the contrary, the spectrum of 3-phenylreumycin **6a** as the anion in basic medium (pH 10, maxima at 298 and 430 nm) was strikingly similar to that of 3-phenyltoxoflavin **5a** in 1,4-dioxane. The UV spectra of 6-phenyl analogue **6b** in these media were also similar to those of 3-phenylreumycin **6a**. For the purpose of the regioselective alkylation at the 1-position of reumycins **6a**,**b** it is clear that the choice of reaction solvent in the basic medium was the most important. We found 1,4-dioxane as the most suitable solvent in the basic medium for the regioselective alkylation. Indeed, we successfully performed the alkylation at the 1-position of reumycins **6a**,**b** in basic medium and 1,4-dioxane, whereupon 1-alkyltoxoflavin derivatives **5a**–**o** were formed. Incidentally, the same alkylation in DMF instead of 1,4-dioxane afforded principally fervenulin derivatives **7a**–**n**.

3-Phenyltoxoflavins **5a**,**h** were prepared by nitrosative cyclisation of the corresponding 6-(2-benzylidene-1-methylhydrazino)-3-methyl (or phenyl)uracils **4b**,**d** according to our previous methods.[6,7] Compounds **5a**,**h** thus obtained were heated in DMF at 140 °C for 5–6 h to afford the desired key intermediates for the alkylation, *i.e.*, 3-phenylreumycins **6a**,**b**.[6,11] Compounds **6a**,**b** were also prepared by nitrosation of 6-benzylidenehydrazino-3-methyl (or phenyl)uracils **4a**,**c**, followed by refluxing the 5-nitroso intermediates formed in acetic anhydride for 1 h.[15]

Table 1 Preparative conditions and yields for toxoflavin **5a**–**o** and fervenulin derivatives **7a**–**n** by alkylation of reumycin derivatives **6a**,**b**

Run	Substrate	Substituents		Alkylating agent	Reaction conditions[a]			Isolated product and yield (%)[b]	
		R^1	R^2		Solvent	Temp/°C	Time/h	Toxoflavin-type	Fervenulin-type
1	**6a**	Me	Me	$(R^2O)_2SO_2$	1,4-Dioxane	120	2	**5a** (91)	N.I.
2	**6a**	Me	Me	$(R^2O)_2SO_2$	DMF	140	2	N.I.	**7a** (89)
3	**6a**	Me	Me	R^2I	1,4-Dioxane	120	2	N.I.	**7a** (80)
4	**6a**	Me	Et	$(R^2O)_2SO_2$	1,4-Dioxane	120	2	**5b** (79)	N.I.
5	**6a**	Me	Et	$(R^2O)_2SO_2$	DMF	140	2	N.I.	**7b** (86)
6	**6a**	Me	Et	R^2I	1,4-Dioxane	120	2	N.I.	**7b** (85)
7	**6a**	Me	Pr	$(R^2O)_2SO_2$	1,4-Dioxane	120	2	**5c** (80)	N.I.
8	**6a**	Me	Pr	$(R^2O)_2SO_2$	DMF	140	2	N.I.	**7c** (78)
9	**6a**	Me	Bu	$(R^2O)_2SO_2$	1,4-Dioxane	120	2	**5d** (82)	N.I.
10	**6a**	Me	Bu	$(R^2O)_2SO_2$	DMF	140	2	N.I.	**7d** (81)
11	**6a**	Me	Cyclopentyl	R^2Br	1,4-Dioxane	120	5	**5e** (81)	N.I.
12	**6a**	Me	Cyclopentyl	R^2Br	DMF	140	5	N.I.	**7e** (65)
13	**6a**	Me	Cyclohexyl	R^2Br	1,4-Dioxane	120	5	**5f** (79)	N.I.
14	**6a**	Me	Cyclohexyl	R^2Br	DMF	140	5	**5f** (33)	**7f** (42)
15	**6a**	Me	Pr^i	R^2Br	1,4-Dioxane	120	3	**5g** (85)	N.I.
16	**6a**	Me	Pr^i	R^2Br	DMF	140	3	N.I.	**7g** (67)
17	**6b**	Ph	Me	$(R^2O)_2SO_2$	1,4-Dioxane	120	2	**5h** (86)	N.I.
18	**6b**	Ph	Me	$(R^2O)_2SO_2$	DMF	140	2	N.I.	**7h** (85)
19	**6b**	Ph	Me	R^2I	1,4-Dioxane	120	2	N.I.	**7h** (78)
20	**6b**	Ph	Et	$(R^2O)_2SO_2$	1,4-Dioxane	120	2	**5i** (76)	N.I.
21	**6b**	Ph	Et	$(R^2O)_2SO_2$	DMF	140	2	N.I.	**7i** (75)
22	**6b**	Ph	Et	R^2I	1,4-Dioxane	120	2	N.I.	**7i** (81)
23	**6b**	Ph	Pr	$(R^2O)_2SO_2$	1,4-Dioxane	120	2	**5j** (88)	N.I.
24	**6b**	Ph	Pr	$(R^2O)_2SO_2$	DMF	140	2	N.I.	**7j** (84)
25	**6b**	Ph	Bu	$(R^2O)_2SO_2$	1,4-Dioxane	120	2	**5k** (82)	N.I.
26	**6b**	Ph	Bu	$(R^2O)_2SO_2$	DMF	140	2	N.I.	**7k** (80)
27	**6b**	Ph	Cyclopentyl	R^2Br	1,4-Dioxane	120	5	**5l** (85)	N.I.
28	**6b**	Ph	Cyclopentyl	R^2Br	DMF	140	5	N.I.	**7l** (72)
29	**6b**	Ph	Cyclohexyl	R^2Br	1,4-Dioxane	120	5	**5m** (81)	N.I.
30	**6b**	Ph	Cyclohexyl	R^2Br	DMF	140	5	**5m** (40)	**7m** (49)
31	**6b**	Ph	Pr^i	R^2Br	1,4-Dioxane	120	3	**5n** (78)	N.I.
32	**6b**	Ph	Pr^i	R^2Br	DMF	140	3	N.I.	**7n** (82)
33	**6b**	Ph	Bu^t	R^2Br	1,4-Dioxane	120	7	**5o** (85)	N.I.
34	**6b**	Ph	Bu^t	R^2Br	DMF	140	9	N.I.	N.I.

[a] All reactions were heated in the presence of anhydrous potassium carbonate as a base (*method A*). [b] N.I. means not isolated.

The primary alkylation of reumycin derivatives **6a**,**b** is described below. As can be seen from runs 1, 4, 7, 9, 17, 20, 23 and 25 in Table 1, a mixture of compound **6a** or **6b**, dialkyl sulfate (3 equiv.) and anhydrous potassium carbonate (2 equiv.) in 1,4-dioxane was heated in a sealed vessel under an atmosphere of argon at 120 °C for 2 h to afford the corresponding 1-alkyltoxoflavin type compounds **5a**–**d**,**h**–**k** in 76–91% yields (*method A*). The above alkylations of reumycins **6a**,**b** with other bases, *e.g.* sodium hydride and sodium hydrogen carbonate, were also accomplished in the same direction to get the toxoflavin derivatives **5a**–**d**,**h**–**k** in 65–88% yields as shown in the Experimental section (*methods B and C*). However, the use of alkyl iodides as the alkylating agent in the above reaction gave not the corresponding 1-alkyltoxoflavin type compounds **5a**,**b**,**h**,**i** but 8-alkylfervenulin type compounds **7a**,**b**,**h**,**i** in 78–85% yields (*method D*, runs 3, 6, 19 and 22). When the same reactions were carried out in DMF instead of 1,4-dioxane at 140 °C for 2 h, the corresponding 8-alkylfervenulin type compounds **7a**–**d**,**h**–**k** formed in 75–89% yields (*method E*, runs 2, 5, 8, 10, 18, 21, 24 and 26). The secondary alkylation is as follows: the similar reaction of reumycins **6a**,**b** with an appropriate alkyl bromide (3 equiv.), *e.g.* cyclopentyl bromide, cyclohexyl bromide and 2-bromopropane, and anhydrous potassium carbonate (2 equiv.) in 1,4-dioxane afforded the corresponding 1-alkyltoxoflavin type compounds **5e**–**g**,**l**–**n** in 78–85% yields (*method F*, runs 11, 13, 15, 27, 29 and 31). In the same manner, the secondary alkylation with sodium hydride or sodium hydrogen carbonate instead of potassium carbonate also yielded the corresponding 1-alkyltoxoflavin type compounds **5e**–**g**,**l**–**n** in 73–87% yields (*methods G and H*). On the other hand, the alkylation of reumycins **6a**,**b** with an appropriate cyclopentyl bromide or 2-bromopropane (3 equiv.) and anhydrous potassium carbonate (2 equiv.) in DMF gave the corresponding 8-alkylfervenulin derivatives **7e**,**g**,**l**,**n** in 65–82% yields (*method I*, runs 12, 16, 28 and 32). In the case of the above reaction with cyclohexyl bromide (3 equiv.) as an alkylating agent, the alkylated product was a mixture of 1-alkyltoxoflavin **5f** (33%), **5m** (40%) and 8-alkylfervenulin derivative **7f** (42%), **7m** (49%), respectively (runs 14 and 30). Finally, the tertiary alkylation is as follows. The reaction of reumycin derivative **6b** with 2-bromo-2-methylpropane (3 equiv.) and anhydrous potassium carbonate (2 equiv.) in 1,4-dioxane at 120 °C for 7 h afforded 1-*tert*-butyltoxoflavin derivative **5o** in 85% yield (*method J*, run 33). When the same reaction was carried out in DMF, no alkylated product was obtained owing to steric hindrance between the carbonyl at the 7-position and the *tert*-butyl group at the 8-position to produce the expected 8-*tert*-butylfervenulin **7o** (run 34). The structures of the products **5a**,**h** and **7a**–**c**,**g** were determined by comparison of IR and ^{1}H NMR spectral data of the authentic samples, respectively, and other new compounds **5** and **7** were assigned on the basis of elemental analyses and satisfactory spectral data as shown in Tables 2 and 3. As a general rule, the ultraviolet spectra of toxoflavin type compounds **5a**–**o** in 1,4-dioxane showed two maximum absorption bands at *ca.* 295 and 435 nm, while those of fervenulin type compounds **7a**–**n** did at *ca.* 280 and 375 nm. That is, bathochromic shifts of about 60 nm at the longer wavelength band for toxoflavins **5a**–**o** were observed in comparison with those for fervenulins **7a**–**n**. The mechanism of the above regioselective alkylations is as follows. The real structure of reumycins **3** and **6** in basic solution may be of toxoflavin type structure rather than the fervenulin type structure as noted in

Table 2 Physical and analytical data for the compounds **5a**–**o** and **7a**–**n**

Compound (Formula)	Mp/°C[a]	TLC (R_f)[e]	ν_{max}(KBr)/cm^{-1}	λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1})[f]	Found (%) (Required)		
					C	H	N
5a $C_{13}H_{11}N_5O_2$	228 (Decomp.)[b]	0.48	1660, 1700 (C=O)	293 (4.29), 434 (3.30)		[b]	
5b $C_{14}H_{13}N_5O_2$	163 (Decomp.)	0.51	1660, 1715 (C=O)	291 (4.35), 436 (3.36)	59.5 (59.4)	4.8 (4.6)	24.8 (24.7)
5c $C_{15}H_{15}N_5O_2$	169 (Decomp.)	0.53	1670, 1715 (C=O)	294 (4.33), 436 (3.35)	60.5 (60.6)	5.2 (5.1)	23.7 (23.6)
5d $C_{16}H_{17}N_5O_2$	172 (Decomp.)	0.57	1665, 1710 (C=O)	294 (4.41), 435 (3.47)	61.85 (61.7)	5.6 (5.5)	22.55 (22.5)
5e $C_{17}H_{17}N_5O_2$	228–230	0.58	1660, 1710 (C=O)	295 (4.38), 436 (3.45)	63.0 (63.15)	5.4 (5.3)	21.6 (21.7)
5f $C_{18}H_{19}N_5O_2$	273–275	0.58	1660, 1715 (C=O)	294 (4.41), 434 (3.51)	63.9 (64.1)	5.6 (5.7)	20.65 (20.8)
5g $C_{15}H_{15}N_5O_2$	252–254	0.50	1665, 1715 (C=O)	294 (4.30), 436 (3.33)	60.9 (60.6)	5.2 (5.1)	23.5 (23.6)
5h $C_{18}H_{13}N_5O_2$	245 (Decomp.)[c]	0.42	1660, 1720 (C=O)	296 (4.37), 434 (3.38)		[c]	
5i $C_{19}H_{15}N_5O_2$	192 (Decomp.)	0.50	1675, 1720 (C=O)	298 (4.41), 436 (3.42)	66.0 (66.1)	4.3 (4.4)	20.2 (20.3)
5j $C_{20}H_{17}N_5O_2$	135 (Decomp.)	0.55	1680, 1720 (C=O)	295 (4.41), 436 (3.36)	66.6 (66.8)	5.0 (4.8)	19.4 (19.5)
5k $C_{21}H_{19}N_5O_2 \cdot 1/4\ H_2O$	178 (Decomp.)	0.57	1670, 1720 (C=O)	294 (4.48), 436 (3.47)	66.8 (66.7)	5.1 (5.2)	18.7 (18.5)
5l $C_{22}H_{19}N_5O_2$	224–226	0.58	1675, 1720 (C=O)	299 (4.48), 436 (3.53)	68.7 (68.6)	5.1 (5.0)	18.35 (18.2)
5m $C_{23}H_{21}N_5O_2$	286–288	0.59	1680, 1720 (C=O)	296 (4.50), 434 (3.60)	68.9 (69.2)	5.1 (5.3)	17.6 (17.5)
5n $C_{20}H_{17}N_5O_2$	237–239	0.55	1675, 1720 (C=O)	297 (4.41), 435 (3.41)	66.7 (66.8)	4.7 (4.8)	19.4 (19.5)
5o $C_{21}H_{19}N_5O_2$	275–277	0.55	1670, 1715 (C=O)	296 (4.43), 436 (3.46)	67.6 (67.55)	5.15 (5.1)	18.9 (18.75)
7a $C_{13}H_{11}N_5O_2$	277–279[d]	0.73	1680, 1730 (C=O)	277 (4.30), 375 (3.41)		[d]	
7b $C_{14}H_{13}N_5O_2$	226–228[d]	0.72	1675, 1725 (C=O)	279 (4.30), 372 (3.48)		[d]	
7c $C_{15}H_{15}N_5O_2$	212–214[d]	0.73	1680, 1730 (C=O)	276 (4.34), 375 (3.33)		[d]	
7d $C_{16}H_{17}N_5O_2$	179–181	0.72	1680, 1730 (C=O)	281 (4.36), 377 (3.46)	61.55 (61.7)	5.5 (5.5)	22.7 (22.5)
7e $C_{17}H_{17}N_5O_2$	211–213	0.76	1685, 1730 (C=O)	280 (4.33), 376 (3.43)	63.0 (63.15)	5.1 (5.3)	21.8 (21.7)
7f $C_{18}H_{19}N_5O_2$	207–209	0.76	1680, 1730 (C=O)	280 (4.38), 374 (3.47)	63.95 (64.1)	5.8 (5.7)	20.7 (20.8)
7g $C_{15}H_{15}N_5O_2$	224–226[d]	0.74	1680, 1730 (C=O)	276 (4.31), 375 (3.32)		[d]	
7h $C_{18}H_{13}N_5O_2$	269–271	0.65	1690, 1735 (C=O)	280 (4.38), 377 (3.44)	65.4 (65.25)	4.2 (3.95)	21.3 (21.1)
7i $C_{19}H_{15}N_5O_2 \cdot 1/2\ H_2O$	242–244	0.65	1690, 1730 (C=O)	281 (4.36), 377 (3.40)	64.45 (64.4)	4.45 (4.55)	19.8 (19.8)
7j $C_{20}H_{17}N_5O_2$	237–239	0.65	1690, 1735 (C=O)	281 (4.29), 376 (3.55)	66.7 (66.8)	4.8 (4.8)	19.35 (19.5)
7k $C_{21}H_{19}N_5O_2$	220–222	0.66	1690, 1735 (C=O)	280 (4.41), 376 (3.49)	67.3 (67.55)	4.9 (5.1)	18.8 (18.8)
7l $C_{22}H_{19}N_5O_2$	247–249	0.76	1690, 1730 (C=O)	282 (4.47), 374 (3.53)	68.3 (68.6)	4.8 (5.0)	18.3 (18.2)
7m $C_{23}H_{21}N_5O_2$	282–284	0.76	1690, 1735 (C=O)	281 (4.49), 374 (3.55)	69.35 (69.2)	5.1 (5.3)	17.6 (17.5)
7n $C_{20}H_{17}N_5O_2$	244–246	0.66	1685, 1730 (C=O)	281 (4.41), 374 (3.44)	66.7 (66.8)	4.7 (4.8)	19.4 (19.5)

[a] All products were recrystallised from 40% aqueous 1,4-dioxane and were obtained as yellow or orange needles. [b] Ref. 7. [c] Ref. 6. [d] Ref. 11. [e] All thin-layer chromatograms (TLC) were obtained by developing in ethyl acetate. [f] All UV spectra were measured in 1,4-dioxane.

the UV spectra. Therefore, the alkylation of reumycins **6** under basic conditions in 1,4-dioxane gave predominantly 1-alkyltoxoflavins **5**. However, the same alkylation in DMF instead of 1,4-dioxane afforded predominantly 8-alkylfervenulins **7** because of the character of facile dealkylation of 1-alkyltoxoflavins **5** produced by the alkylation in heating DMF. By the way, the 1-alkyltoxoflavins **5** were stable for more than 12 hours on heating in 1,4-dioxane, water and ethanol.

It is necessary to evaluate the extent of dealkylation of 1-alkyltoxoflavin type derivatives **5** for their chemical stabilities. Table 4 shows the transalkylation from toxoflavin derivatives **5a**–**o** into nucleophiles such as DMF and *n*-butylamine. Thus the reactions were carried out by heating compounds **5a**–**o** (3 mmol) with DMF (10 cm^3) at 140 °C for 3 h and by heating compounds **5d**–**g**,**k**–**o** (3 mmol) with a mixture of *n*-butylamine (1 cm^3) and 1,4-dioxane (10 cm^3) at 100 °C for 1 h. The dealkylation of 3-phenyltoxoflavins **5a**–**d** in DMF was readily observed (runs 1–4), while the rate of dealkylation of 3,6-diphenyltoxoflavins **5h**–**k** was apparently retarded with increasing the alkyl chain length at the 1-position (runs 5–8). On the other hand, the dealkylation of compounds **5e**–**g**,**l**–**o** possessing a secondary or tertiary alkyl group was not observed

Table 3 ^{1}H NMR spectroscopic data for the compounds **5a**–**o** and **7a**–**n**

Compound	δ_H [200 MHz; $(CD_3)_2SO$; Me_4Si]
5a[a]	3.29 (3 H, s, 6-Me), 4.06 (3 H, s, 1-Me), 7.53–7.65 (3 H, m, Ph-*m*,*p*H), 8.08–8.28 (2 H, m, Ph-*o*H)
5b[a]	1.48 (3 H, t, *J* 7.0, CH_2CH_3), 3.29 (3 H, s, 6-Me), 4.50 (2 H, q, *J* 7.0, CH_2CH_3), 7.50–7.72 (3 H, m, Ph-*m*,*p*H), 8.15–8.30 (2 H, m, Ph-*o*H)
5c[a]	1.00 (3 H, t, *J* 7.0, $[CH_2]_2CH_3$), 1.97 (2 H, pseudosextet, $CH_2CH_2CH_3$), 3.29 (3 H, s, 6-Me), 4.42 (2 H, t, *J* 7.0, $CH_2CH_2CH_3$), 7.50–7.70 (3 H, m, Ph-*m*,*p*H), 8.12–8.31 (2 H, m, Ph-*o*H)
5d	0.95 (3 H, t, *J* 7.3, $[CH_2]_3CH_3$), 1.41 (2 H, sex, *J* 7.3, $[CH_2]_2CH_2CH_3$), 1.90 (2 H, quin, *J* 7.4, $CH_2CH_2CH_2CH_3$), 3.27 (3 H, s, 6-Me), 4.45 (2 H, t, *J* 7.2, $CH_2[CH_2]_2CH_3$), 7.58–7.62 (3 H, m, Ph-*m*,*p*H), 8.18–8.21 (2 H, m, Ph-*o*H)
5e	1.70–2.26 (8 H, m, cyclopentyl-H), 3.27 (3 H, s, 6-Me), 5.67 (1 H, pseudoquintet, cyclopentyl-H), 7.55–7.64 (3 H, m, Ph-*m*,*p*H), 8.14–8.23 (2 H, m, Ph-*o*H)
5f	1.13–2.06 (10 H, m, cyclohexyl-H), 3.27 (3 H, s, 6-Me), 5.10–5.28 (1 H, m, cyclohexyl-H), 7.56–7.66 (3 H, m, Ph-*m*,*p*H), 8.18–8.27 (2 H, m, Ph-*o*H)
5g[a]	1.50 (6 H, d, *J* 6.5, $CH[CH_3]_2$), 3.29 (3 H, s, 6-Me), 5.55 (1 H, quin, *J* 6.5, $CH[CH_3]_2$), 7.50–7.70 (3 H, m, Ph-*m*,*p*H), 8.15–8.37 (2 H, m, Ph-*o*H)
5h	4.11 (3 H, s, 1-Me), 7.19–7.29 (2 H, m, 6-Ph-*m*H), 7.41–7.66 (6 H, 3-Ph-*m*,*p*H and 6-Ph-*o*,*p*H), 8.17–8.27 (2 H, m, 3-Ph-*o*H)
5i[a]	1.52 (3 H, t, *J* 7.1, CH_2CH_3), 4.55 (2 H, q, *J* 7.1, CH_2CH_3), 7.16–7.34 (2 H, m, 6-Ph-*m*H), 7.42–7.65 (6 H, m, 3-Ph-*m*,*p*H and 6-Ph-*o*,*p*H), 8.12–8.34 (2 H, m, 3-Ph-*o*H)
5j	0.98 (3 H, t, *J* 7.3, $[CH_2]_2CH_3$), 1.86 (2 H, sex, *J* 7.3, $CH_2CH_2CH_3$), 3.96 (2 H, t, *J* 7.1, $CH_2CH_2CH_3$), 7.46–7.55 (4 H, m, 3-Ph-*p*H and 6-Ph-*m*,*p*H), 7.57–7.67 (2 H, m, 3-Ph-*m*H), 7.69–7.76 (2 H, m, 6-Ph-*o*H), 8.21–8.29 (2 H, m, 3-Ph-*o*H)
5k	0.95 (3 H, t, *J* 7.3, $[CH_2]_3CH_3$), 1.42 (2 H, sex, *J* 7.3, $[CH_2]_2CH_2CH_3$), 1.83 (2 H, quin, *J* 7.4, $CH_2CH_2CH_2CH_3$), 4.01 (2 H, t, *J* 7.1, $CH_2[CH_2]_2CH_3$), 7.48–7.55 (4 H, m, 3-Ph-*p*H and 6-Ph-*m*,*p*H), 7.58–7.67 (2 H, m, 3-Ph-*m*H), 7.70–7.76 (2 H, m, 6-Ph-*o*H), 8.21–8.29 (2 H, m, 3-Ph-*o*H)
5l	1.70–2.30 (8 H, m, cyclopentyl-H), 5.74 (1 H, pseudoquintet, cyclopentyl-H), 7.20–7.27 (2 H, m, 6-Ph-*m*H), 7.38–7.66 (6 H, m, 3-Ph-*m*,*p*H and 6-Ph-*o*,*p*-H), 8.14–8.26 (2 H, m, 3-Ph-*o*H)
5m	1.20–2.15 (10 H, m, cyclohexyl-H), 5.10–5.30 (1 H, m, cyclohexyl-H), 7.17–7.27 (2 H, m, 6-Ph-*m*H), 7.42–7.57 (6 H, m, 3-Ph-*m*,*p*H and 6-Ph-*o*,*p*H), 8.15–8.29 (2 H, m, 3-Ph-*o*H)
5n[a]	1.54 (6 H, d, *J* 6.5, $CH[CH_3]_2$), 5.62 (1 H, quin, *J* 6.5, $CH[CH_3]_2$), 7.15–7.34 (2 H, m, 6-Ph-*m*H), 7.42–7.66 (6 H, m, 3-Ph-*m*,*p*H and 6-Ph-*o*,*p*-H), 8.14–8.38 (2 H, m, 3-Ph-*o*H)
5o[a]	1.89 (9 H, s, 3 × Me), 7.23–7.70 (8 H, m, 3-Ph-*m*,*p*H and 6-Ph-*o*,*m*,*p*H), 8.14–8.36 (2 H, m, 3-Ph-*o*H)
7a	3.37 (3 H, s, 6-Me), 3.73 (3 H, s, 8-Me), 7.54–7.80 (3 H, m, Ph-*m*,*p*H), 8.34–8.56 (2 H, m, Ph-*o*H)
7b[a]	1.32 (3 H, t, *J* 7.0, CH_2CH_3), 3.37 (3 H, s, 6-Me), 4.42 (2 H, q, *J* 7.0, CH_2CH_3), 7.57–7.76 (3 H, m, Ph-*m*,*p*H), 8.35–8.57 (2 H, m, Ph-*o*H)
7c	0.97 (3 H, t, *J* 7.4, $[CH_2]_2CH_3$), 1.94 (2 H, sex, *J* 7.3, $CH_2CH_2CH_3$), 3.35 (3 H, s, 6-Me), 4.31 (2 H, t, *J* 7.2, $CH_2CH_2CH_3$), 7.58–7.64 (3 H, m, Ph-*m*,*p*H), 8.39–8.46 (2 H, m, Ph-*o*H)
7d	0.94 (3 H, t, *J* 7.3, $[CH_2]_3CH_3$), 1.41 (2 H, sex, *J* 7.3, $[CH_2]_2CH_2CH_3$), 1.71 (2 H, pseudoquintet, $CH_2CH_2CH_2CH_3$), 3.35 (3 H, s, 6-Me), 4.35 (2 H, t, *J* 7.3, $CH_2[CH_2]_2CH_3$), 7.58–7.64 (3 H, m, Ph-*m*,*p*H), 8.38–8.46 (2 H, m, Ph-*o*H)
7e	1.60–1.75 (2 H, m, cyclopentyl-H), 1.88–2.10 (4 H, m, cyclopentyl-H), 2.14–2.30 (2 H, m, cyclopentyl-H), 3.34 (3 H, s, 6-Me), 5.86 (1 H, pseudoquintet, cyclopentyl-H), 7.59–7.64 (3 H, m, Ph-*m*,*p*H), 8.38–8.45 (2 H, m, Ph-*o*H)
7f	1.18–1.56 (3 H, m, cyclohexyl-H), 1.65–1.96 (5 H, m, cyclohexyl-H), 2.40–2.63 (2 H, m, cyclohexyl-H), 3.33 (3 H, s, 6-Me), 5.31 (1 H, br pseudotriplet, cyclohexyl-H), 7.58–7.67 (3 H, m, Ph-*m*,*p*H), 8.38–8.47 (2 H, m, Ph-*o*H)
7g[a]	1.61 (6 H, d, *J* 7.0, $CH[CH_3]_2$), 3.35 (3 H, s, 6-Me), 5.65 (1 H, quin, *J* 7.0, $CH[CH_3]_2$), 7.55–7.70 (3 H, m, Ph-*m*,*p*H), 8.34–8.50 (2 H, m, Ph-*o*H)
7h[a]	3.75 (3 H, s, 8-Me), 7.23–7.74 (8 H, m, 3-Ph-*m*,*p*H and 6-Ph-*o*,*m*,*p*H), 8.36–8.57 (2 H, m, 3-Ph-*o*H)
7i[a]	1.36 (3 H, t, *J* 7.0, CH_2CH_3), 4.44 (2 H, q, *J* 7.0, CH_2CH_3), 7.23–7.76 (8 H, m, 3-Ph-*m*,*p*H and 6-Ph-*o*,*m*,*p*H), 8.34–8.60 (2 H, m, 3-Ph-*o*H)
7j	0.99 (3 H, t, *J* 7.4 $[CH_2]_2CH_3$), 1.79 (2 H, sex, *J* 7.3, $CH_2CH_2CH_3$), 4.34 (2 H, t, *J* 7.3, $CH_2CH_2CH_3$), 7.30–7.36 (2 H, m, 6-Ph-*m*H), 7.46–7.56 (3 H, m, 6-Ph-*o*,*p*H), 7.60–7.66 (3 H, m, 3-Ph-*m*,*p*H), 8.41–8.48 (2 H, m, 3-Ph-*o*H)
7k	0.94 (3 H, t, *J* 7.3, $[CH_2]_3CH_3$), 1.43 (2 H, sex, *J* 7.3, $[CH_2]_2CH_2CH_3$), 1.75 (2 H, pseudoquintet, $CH_2CH_2CH_2CH_3$), 4.37 (2 H, t, *J* 7.3, $CH_2[CH_2]_2CH_3$), 7.30–7.37 (2 H, m, 6-Ph-*m*H), 7.47–7.56 (3 H, m, 6-Ph-*o*,*p*H), 7.60–7.68 (3 H, m, 3-Ph-*m*,*p*H), 8.41–8.49 (2 H, m, 3-Ph-*o*H)
7l	1.55–1.74 (2 H, m, cyclopentyl-H), 1.87–2.07 (4 H, m, cyclopentyl-H), 2.14–2.35 (2 H, m, cyclopentyl-H), 5.88 (1 H, br pseudotriplet, cyclopentyl-H), 7.30–7.39 (2 H, m, 6-Ph-*m*H), 7.47–7.70 (6 H, m, 3-Ph-*m*,*p*H and 6-Ph-*o*,*p*H), 8.40–8.52 (2 H, m, 3-Ph-*o*H)
7m	1.15–1.55 (3 H, m, cyclohexyl-H), 1.62–1.95 (5 H, m, cyclohexyl-H), 2.40–2.60 (2 H, m cyclohexyl-H), 5.31 (1 H, br pseudotriplet), 7.29–7.37 (2 H, m, 3-Ph-*m*H), 7.46–7.56 (3 H, m, 6-Ph-*o*,*p*H), 7.60–7.66 (3 H, m, 3-Ph-*m*,*p*H), 8.41–8.48 (2 H, m, 3-Ph-*o*H)
7n[a]	1.61 (6 H, d, *J* 7.0, $CH[CH_3]_2$), 5.65 (1 H, quin, *J* 7.0, $CH[CH_3]_2$), 7.22–7.80 (8 H, m, 3-Ph-*m*,*p*H and 6-Ph-*o*,*m*,*p*H), 8.34–8.60 (2 H, m, 3-Ph-*o*H)

[a] This compound was measured at 60 MHz.

under these conditions (runs 9–15). However, the dealkylation of compounds **5d**–**g**,**k**–**o**, even compound **5o** possessing a *tert*-alkyl group, by *n*-butylamine as a stronger nucleophile took place easily, and the nucleophile itself is presumably alkylated by the alkyl groups eliminated (runs 16–24). Previously, we have reported a possible reaction mechanism as follows. That was, toxoflavin **1** and its 3-substituted derivatives readily underwent demethylation with several nucleophiles *e.g.* DMF and dimethylacetamide, to give the corresponding 1-demethyltoxoflavin (reumycin) **3** and its 3-substituted derivatives and during the reactions novel radical species, *i.e.* toxoflavin radical anions, were observed.[10] On the other hand, DMF or dimethylacetamide could be methylated into reactive dimethylformamide ether or dimethylacetamide ether, which is readily hydrolysed into methanol and the original solvents.

Conclusion

Thus, we reported the first successful synthesis of 1-substituted toxoflavin derivatives **5** by the regioselective alkylation of reumycins **6**, and this simple methodology provided a facile and convenient route to the preparation of 1-alkyltoxoflavins **5** which are biologically more active than 8-alkylfervenulins **7**. Moreover, the rates of transalkylation from 1-alkyltoxoflavin derivatives **5** into nucleophiles such as DMF and *n*-butylamine to produce reumycins **6** were also determined.

Experimental

General

Mps were obtained on a Yanagimoto micro melting point apparatus and were uncorrected. Microanalyses were measured

Table 4 Transalkylation from toxoflavins **5a**–**o** into nucleophiles such as DMF and *n*-butylamine to produce reumycins **6a**,**b**

Run	Starting material			Nucleophile[a]	Proportion of analysed materials **5**:**6** (%)[b]
		R^1	R^2		
1	**5a**	Me	Me	DMF	0:100 (91)
2	**5b**	Me	Et	DMF	0:100 (93)
3	**5c**	Me	Pr	DMF	0:100 (89)
4	**5d**	Me	Bu	DMF	0:100 (86)
5	**5h**	Ph	Me	DMF	0:100 (90)
6	**5i**	Ph	Et	DMF	10: 90 (95)
7	**5j**	Ph	Pr	DMF	10: 90 (91)
8	**5k**	Ph	Bu	DMF	50: 50 (88)
9	**5e**	Me	Cyclopentyl	DMF	100: 0
10	**5f**	Me	Cyclohexyl	DMF	100: 0
11	**5g**	Me	Pr^i	DMF	100: 0
12	**5l**	Ph	Cyclopentyl	DMF	100: 0
13	**5m**	Ph	Cyclohexyl	DMF	100: 0
14	**5n**	Ph	Pr^i	DMF	100: 0
15	**5o**	Ph	Bu^t	DMF	100: 0
16	**5d**	Me	Bu	$BuNH_2$	0:100 (86)
17	**5e**	Me	Cyclopentyl	$BuNH_2$	0:100 (88)
18	**5f**	Me	Cyclohexyl	$BuNH_2$	0:100 (84)
19	**5g**	Me	Pr^i	$BuNH_2$	0:100 (87)
20	**5k**	Ph	Bu	$BuNH_2$	0:100 (83)
21	**5l**	Ph	Cyclopentyl	$BuNH_2$	0:100 (85)
22	**5m**	Ph	Cyclohexyl	$BuNH_2$	0:100 (82)
23	**5n**	Ph	Pr^i	$BuNH_2$	0:100 (80)
24	**5o**	Ph	Bu^t	$BuNH_2$	50: 50 (78)

[a] The dealkylation in DMF was carried out at 140 °C for 3 h, while in *n*-butylamine–1,4-dioxane (1:10) it was carried out at 100 °C for 1 h. [b] The yields given in parentheses represent isolated total yields.

on a Yanaco CHN Corder MT-5 apparatus. IR spectra were recorded on a JASCO IRA-102 spectrometer. Ultraviolet (UV) spectra were recorded with a Beckman DU-68 spectrophotometer. ^{1}H NMR spectra were obtained using Hitachi FT-NMR R-1500 (60 MHz) and Varian VXR 200 MHz spectrometers. In all cases, chemical shifts are in δ (ppm) relative to TMS as internal standards, *J*-values are given in Hz, and signals are quoted as follows: s, singlet, d, doublet; t, triplet; q, quartet; quin, quintet; sex, sextet; br, broad; m, multiplet. All reagents were of commercial quality from freshly opened containers and were used without further purification. Organic solvents were dried by standard methods and distilled before use. Reaction progress was monitored by analytical thin layer chromatography (TLC) on pre-coated aluminium-backed plates (Merck Kieselgel 60 F_{254}) using ethyl acetate as the developing solvent and products were visualised by UV light. Column chromatography was run on Kiesel gel 60 (70–230 mesh ASTM, Merck) and eluted with benzene–EtOAc (9:1). All reactions in a sealed vessel were carried out under an atmosphere of argon and all products were recrystallised from 1,4-dioxane, unless otherwise noted. The reaction temperatures are indicated as the temperature of the oil bath.

Transformation of 1-methyl-3,6-diphenylpyrimido[5,4-*e*][1,2,4]-triazine-5,7(1*H*,6*H*)-dione 5h (*toxoflavin type*) into 8-methyl-3,6-diphenylpyrimido[5,4-*e*][1,2,4]triazine-5,7(6*H*,8*H*)-dione 7h (*fervenulin type*)

A stirring mixture of toxoflavin analogue **5h**[6] (0.66 g, 2.1 mmol), methyl iodide (1.42 g, 10.0 mmol) and anhydrous potassium carbonate (0.7 g, 5.1 mmol) in DMF (50 cm^3) was heated at 140 °C for 2 hours. After cooling, the precipitated potassium carbonate was filtered off and the filtrate was concentrated *in vacuo*. A solution of the residue in water (100 cm^3) was extracted with ethyl acetate (3 × 30 cm^3) and the combined extracts were dried over anhydrous $MgSO_4$. Then the extract was evaporated *in vacuo* to leave a solid, which was recrystallised to afford the pure *fervenulin derivative* **7h** (0.43 g, 65%) as yellow needles and shown in Tables 2 and 3.

6-Methyl-3-phenylpyrimido[5,4-*e*][1,2,4]triazine-5,7(6*H*,8*H*)-dione (*3-phenylreumycin*) 6a

A solution of 3-phenyltoxoflavin **5a**[7] (5.0 g, 18.6 mmol) in DMF (70 cm^3) was heated at 140 °C for 5 hours. Concentration of the solution *in vacuo* and recrystallisation of the residue from EtOH gave *3-phenylreumycin* **6a** (4.1 g, 86%), which was identical with an authentic sample,[11] as pale green crystals, mp > 320 °C; R_f (EtOAc) 0.59; ν_{max}/cm^{-1} 3170 (NH), 1735 and 1670 (C=O); δ_H [200 MHz; $(CD_3)_2SO$] 3.30 (3 H, s, 6-Me), 7.58–7.66 (3 H, m, Ph-*m*,*p*H), 8.37–8.45 (2 H, m, Ph-*o*H) and 12.48 (1 H, s, 8-H); λ_{max} (1,4-dioxane)/nm 271 (log ε 4.26) and 367 (3.42); λ_{max} (pH 1 buffer)/nm 270 (log ε 4.21) and 367 (3.11); λ_{max} (pH 10 buffer)/nm 298 (log ε 4.19) and 430 (3.39).

3,6-Diphenylpyrimido[5,4-*e*][1,2,4]triazine-5,7(6*H*,8*H*)-dione (*3,6-diphenylreumycin*) 6b

A solution of 3,6-diphenyltoxoflavin **5h**[6] (10.0 g, 30.2 mmol) in DMF (120 cm^3) was heated at 140 °C for 6 hours. Concentration of the solution *in vacuo* and recrystallisation of the residue gave *3,6-diphenylreumycin* **6b** (7.9 g, 82%), which was identical with an authentic sample,[6] as yellow crystals, mp 328–330 °C; R_f (EtOAc) 0.64; ν_{max}/cm^{-1} 3420 (NH), 1735 and 1690 (C=O); δ_H [60 MHz; $(CD_3)_2SO$] 7.21–7.78 (8 H, m, 3-Ph-*m*,*p*H and 6-Ph-*o*,*m*,*p*H), 8.36–8.52 (2 H, m, 3-Ph-*o*H) and 12.96 (1 H, s, 8-NH); λ_{max} (1,4-dioxane)/nm 276 (log ε 4.40) and 373 (3.52); λ_{max} (pH 1 buffer)/nm 277 (log ε 4.39) and 372 (3.22); λ_{max} (pH 10 buffer)/nm 294 (log ε 4.40) and 413 (3.54).

Regioselective alkylation of reumycin derivatives 6a,b by primary alkylating agents

Toxoflavin derivatives 5a–d,h–k; general procedure. *Method A.* To reumycin **6a** or **6b** (2.0 mmol) and anhydrous potassium carbonate (0.55 g, 4.0 mmol) in 1,4-dioxane (50 cm^3) was added an appropriate dialkyl sulfate (6.0 mmol) and the stirring mixture was heated under reflux at 120 °C for 2 hours. After cooling, the precipitated potassium carbonate was filtered off and the filtrate was concentrated *in vacuo*. A solution of the residue in water (100 cm^3) was extracted with ethyl acetate (3 × 30 cm^3) and the combined extracts were dried over anhydrous $MgSO_4$. Then the extract was evaporated *in vacuo* to leave a solid, which was recrystallised to afford the corresponding pure *toxoflavin derivatives* **5a**–**d**,**h**–**k** as shown in Tables 1 (runs 1, 4, 7, 9, 17, 20, 23 and 25), 2 and 3.

Method B. To reumycin **6a** or **6b** (2.0 mmol) and 60% sodium hydride (oil dispersion, 0.16 g, 4.0 mmol) in 1,4-dioxane (50 cm^3) was added an appropriate dialkyl sulfate (6.0 mmol) and the mixture was stirred at room temperature for 10–20 hours. After the reaction monitored by TLC was complete, the precipitated sodium hydride was filtered off and the filtrate was concentrated *in vacuo*. A solution of the residue in water (100 cm^3) was extracted with ethyl acetate (3 × 30 cm^3) and the combined extracts were dried over anhydrous $MgSO_4$. Then the extract was evaporated *in vacuo* to leave a solid, which was washed with diethyl ether and recrystallised to afford the corresponding pure *toxoflavin derivatives* **5a** (81), **5b** (85), **5c** (79), **5d** (76), **5h** (72), **5i** (75), **5j** (65) and **5k** (70%).

Method C. The same alkylations of reumycin **6a** or **6b** (2.0 mmol), with sodium hydrogen carbonate (0.34 g, 4.0 mmol) instead of anhydrous potassium carbonate, as *method A* afforded the corresponding *toxoflavin derivatives* **5a** (88), **5b** (86), **5c** (76), **5d** (81), **5h** (77), **5i** (76), **5j** (82) and **5k** (78%).

Fervenulin derivatives 7a–d,h–k; general procedure. *Method D.* To reumycin **6a** or **6b** (2.0 mmol) and anhydrous potassium carbonate (0.55 g, 4.0 mmol) in 1,4-dioxane or DMF (50 cm^3) was added an appropriate alkyl iodide (10.0 mmol) and the stirring mixture was heated at 120 or 140 °C for 2 hours. After the same work-up as noted in *method A*, a recrystallisation of the crude crystals gave the corresponding pure *fervenulin derivatives* **7a**,**b**,**h**,**i** as shown in Tables 1 (runs 3, 6, 19 and 22), 2 and 3.

Method E. The same alkylations of reumycin **6a** or **6b** (2.0 mmol), in DMF (50 cm^3) instead of in 1,4-dioxane, as *method A* afforded the corresponding *fervenulin derivatives* **7a**–**d**,**h**–**k** as shown in Tables 1 (runs 2, 5, 8, 10, 18, 21, 24 and 26), 2 and 3.

Regioselective alkylation of reumycin derivatives 6a,b by secondary alkylating agents

Toxoflavin derivatives 5e–g,l–n; general procedure. *Method F.* To reumycin **6a** or **6b** (2.0 mmol) and anhydrous potassium carbonate (0.55 g, 4.0 mmol) in 1,4-dioxane (50 cm^3) was added an appropriate cyclopentyl bromide, cyclohexyl bromide or 2-bromopropane (6.0 mmol) and the stirring mixture was heated under reflux at 120 °C for 3–5 hours. After the same work-up as noted in *method A*, recrystallisation of the crude crystals gave the corresponding pure *toxoflavin derivatives* **5e**–**g**,**l**–**n** as shown in Tables 1 (runs 11, 13, 15, 27, 29 and 31), 2 and 3.

Method G. To reumycin **6a** or **6b** (2.0 mmol) and 60% sodium hydride (oil dispersion, 0.16 g, 4.0 mmol) in 1,4-dioxane (50 cm^3) was added an appropriate cyclopentyl bromide, cyclohexyl bromide or 2-bromopropane (6.0 mmol) and the mixture was stirred at room temperature for 10–24 hours. After the same work-up as noted in *method B*, recrystallisation of the crude crystals gave the corresponding pure *toxoflavin derivatives* **5e** (73), **5f** (73), **5g** (83), **5l** (76), **5m** (79) and **5n** (76%).

Method H. The same alkylations of reumycin **6a** or **6b** (2.0 mmol) with sodium hydrogen carbonate (0.34 g, 4.0 mmol) instead of anhydrous potassium carbonate as the *method F* afforded corresponding *toxoflavin derivatives* **5e** (81), **5f** (80), **5g** (87), **5l** (78), **5m** (76) and **5n** (83%).

Fervenulin derivatives 7e–g,l–n; general procedure. *Method I.* To reumycin **6a** or **6b** (2.0 mmol) and anhydrous potassium carbonate (0.55 g, 4.0 mmol) in DMF (50 cm^3) was added an appropriate cyclopentyl bromide, cyclohexyl bromide or 2-bromopropane (6.0 mmol) and the stirring mixture was heated at 140 °C for 3–5 hours. After the same work-up as noted in *method A*, recrystallisation of the crude crystals gave the corresponding pure *fervenulin derivatives* **7e**,**g**,**l**,**n** as shown in Tables 1 (runs 12, 16, 28 and 32), 2 and 3. In the case of the reaction with cyclohexyl bromide as an alkylating agent, the residue obtained by concentration of the extract *in vacuo* was subject to column chromatography to isolate the corresponding pure *toxoflavin derivatives* **5f**,**m** and *fervenulin derivatives* **7f**,**m** from the mixture of two products, respectively, as shown in Tables 1 (runs 14 and 30), 2 and 3.

Regioselective alkylation of reumycin derivatives 6b by tertiary alkylating agent

1-*tert*-Butyl-3,6-diphenylpyrimido[5,4-*e*][1,2,4]triazine-5,7-(1*H*,6*H*)-dione (*toxoflavin derivative*) 5o. *Method J.* To reumycin **6b** (0.635 g, 2.0 mmol) and anhydrous potassium carbonate (0.55 g, 4.0 mmol) in 1,4-dioxane (50 cm^3) was added 2-bromo-2-methylpropane (0.82 g, 6.0 mmol) and the stirring mixture was heated under reflux at 120 °C for 7 hours. After the same work-up as noted in *method A*, recrystallisation of the crude crystals afforded the corresponding pure *1-tert-butyltoxoflavin derivative* **5o** as shown in Tables 1 (run 33), 2 and 3. In the case of the same reaction in DMF as the solvent, no alkylation product was obtained as shown in Table 1 (run 34).

Transalkylation from toxoflavin derivatives (5a–o) into DMF (dealkylation of toxoflavin derivatives). A stirring solution of an appropriate 3-phenyltoxoflavin derivatives **5a**–**g** (3.0 mmol) in DMF (10 cm^3) was heated at 140 °C for 3 hours. After heating, the solution was evaporated *in vacuo* and the residue was recrystallised from EtOH or 40% aqueous 1,4-dioxane to afford the corresponding *3-phenylreumycin* **6a** or starting materials **5e**–**g** in high yields as shown in Table 4 (runs 1–4 and 9–11). On the other hand, in the reaction of 3,6-diphenyltoxoflavin derivatives **5h**–**o** in the same reaction conditions, the reaction residue evaporated was triturated with a small amount of ethanol or 1,4-dioxane to isolate the corresponding *3,6-diphenylreumycin derivative* **6b** or starting materials **5l**–**o** (runs 5 and 12–15). In the case of no single compound being obtained, the residue was subject to column chromatography to isolate the corresponding *3,6-diphenylreumycin derivative* **6b** and starting materials **5i**–**k**, respectively (runs 6–8).

Transalkylation from toxoflavin derivatives (5d–g,k–o) into *n*-butylamine (dealkylation of toxoflavin derivatives). A stirring solution of an appropriate toxoflavin derivative **5d**–**g**,**k**–**o** (3.0 mmol) with *n*-butylamine (1.0 cm^3) in 1,4-dioxane (10.0 cm^3) was heated at 100 °C for one hour. After heating, the solution was evaporated *in vacuo* and the residue was triturated with EtOH to afford the corresponding *reumycin derivatives* **6a**,**b** in high yields as shown in Table 4 (runs 16–23). In the case of the reaction of 1-*tert*-butyltoxoflavin derivative **5o**, the residue was subject to column chromatography to isolate the *reumycin derivative* **6b** and starting material **5o** (run 24).

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