Studies on β-Lactam Antibiotics

Synthesis and Antibacterial Activity of Novel C-3 Alkyne-substituted Cephalosporins

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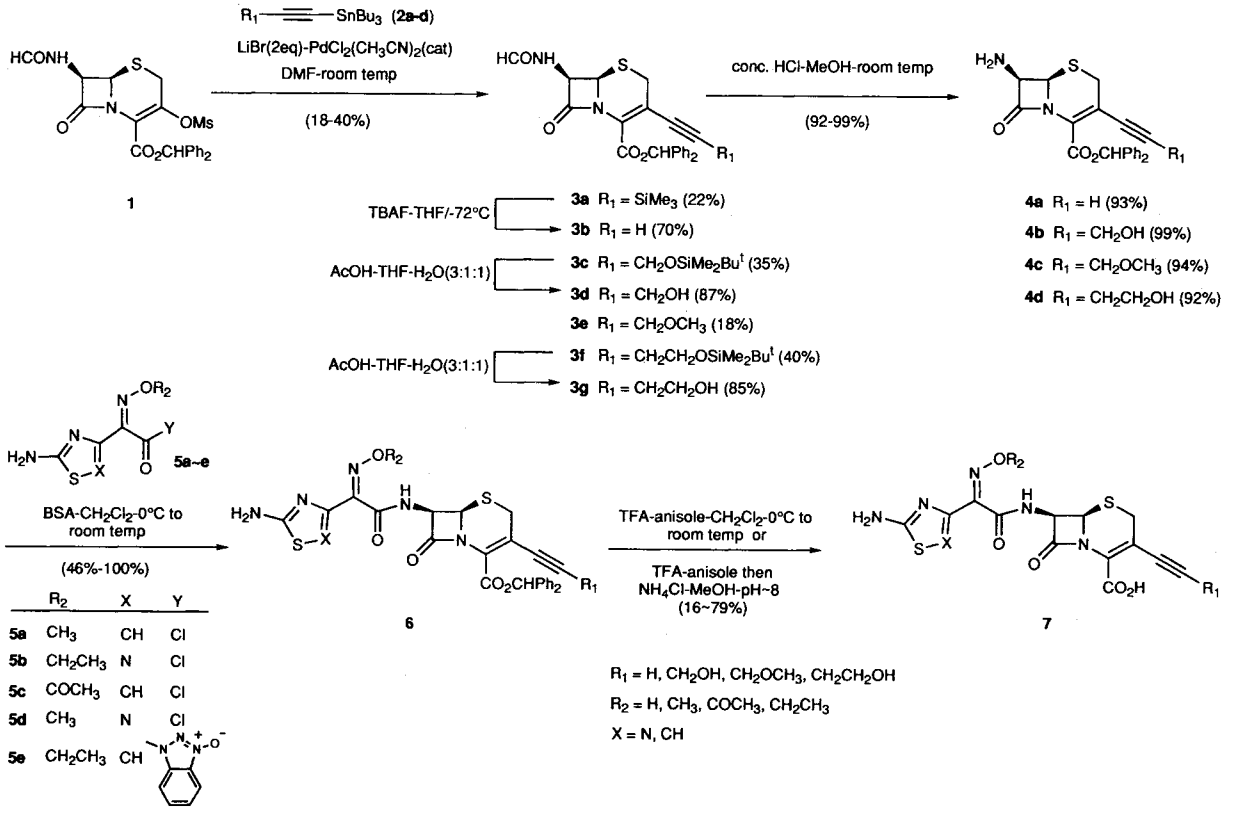
Semi-synthetic modification of the cephalosporin nucleus has provided several generations of innovative, and clinically significant anti-infective agents.[1] Of particular interest, the introduction of a direct C-3 olefinic linkage led to the discovery of cefixime and cefdinir, potent orally absorbed agents that enjoy widespread clinical application.[2~4] As part of the study of structure activity relationships of C-3 carbon linked substituents, we earlier reported the synthesis and biological evaluation of several 3-ethynylcephalosporins.[5,6] In particular, carboxymethoxyimino derivative **7b**, the alkyne analog of cefixime, was characterized by good antibacterial activity against both Gram-negative and Gram-positive bacteria and was orally absorbed.[5] In contrast, hydroxyimino derivative **7a**, the alkyne analog of cefdinir, had reduced antibacterial activity and very low oral absorption.[6] In a continuation of research into the synthesis of novel C-3 carbon linked derivatives, we wish to disclose the synthesis and antibacterial evaluation of novel substituted alkynes, including several bearing hydrophilic substituents, that had improved antibacterial activity in comparison to the previously reported alkynes **7a** and **7b**.

Our strategy for the synthesis of the novel alkynes prepared in this work is outlined in Scheme 1. Whilst we reported earlier[5] that alkyne **3b** could be obtained smoothly from the corresponding cephalosporin-3-formyl derivatives by application of the well known Corey-Fuchs alkynation process *via* 2,2-dibromoolefins, attempts to derive higher analogs by anion generation and alkylation of **3b** were unsuccessful. However, application of the Stille C-C bond-forming process described by FARINA *et al.* at Bristol-Myers,[7,8] whereby activated 3-functionalized cephems undergo coupling with a variety of stannanes in the presence of palladium catalysts, provided us with a very direct synthesis of our target compounds.

Whilst very few examples of couplings with alkynylstannanes have been reported, we found that 'ligandless' palladium-catalysed coupling[9] of the stannanes **2a~2d**

Scheme 1. Synthesis of novel C-3 alkyne-substituted cephalosporins.



	R_2	X	Y
5a	CH_3	CH	Cl
5b	CH_2CH_3	N	Cl
5c	$COCH_3$	CH	Cl
5d	CH_3	N	Cl
5e	CH_2CH_3	CH	

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Scheme 2. Synthesis of tributyltin reagents.

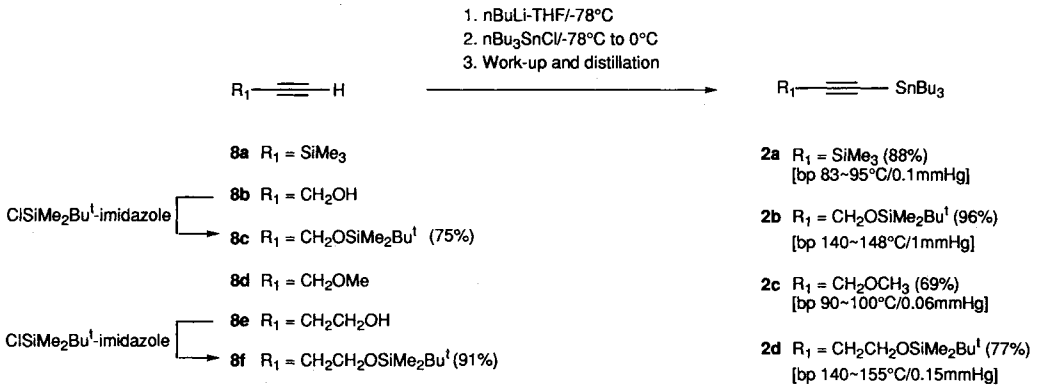

Table 1. Yields of **6**, **7** and *in vitro* antibacterial activity of novel C-3-alkyne-substituted cephalosporins **7**.

Compound No.	Yield of **6** (%)	Yield of **7** (%)	R_1	R_2	X	MIC (μg/ml)				
						S.a.	*E.c.*	*K.p.*	*P.m.*	*P.v.*[a]
7a[6]	N/A	N/A	H	H	CH	3.13	1.56	1.56	0.78	12.5
7b[5]	N/A	N/A	H	CH_2CO_2H	CH	6.25	1.56	0.39	0.05	0.05
7c	98	59	H	CH_3	CH	3.13	0.39	0.39	0.05	≤0.025
7d	88	26	H	CH_2CH_3	N	6.25	0.78	0.78	0.2	0.1
7e	94	79	CH_2OH	$COCH_3$	CH	0.39	0.78	0.78	0.2	0.78
7f	100	77	CH_2OH	CH_3	CH	3.13	0.78	0.78	0.1	0.1
7g	86	63	CH_2OH	CH_3	N	3.13	0.39	0.39	0.1	0.2
7h	86	63	CH_2OH	CH_2CH_3	N	1.56	0.39	0.78	0.2	0.05
7i	100	40	CH_2OCH_3	H	CH	0.78	0.39	0.39	0.1	0.78
7j	100	26	CH_2OCH_3	CH_3	CH	3.13	0.2	0.39	≤0.025	0.05
7k[c]	100	43	CH_2CH_2OH	H	CH	0.39	0.39	0.78	0.1	0.39
7l[d]	100	64	CH_2CH_2OH	CH_3	CH	3.13	0.39	0.78	0.05	0.05
7m[e]	49	80	CH_2CH_2OH	CH_2CH_3	CH	3.13	0.78	1.56	0.2	≤0.025
7n	96	76	CH_2CH_2OH	CH_3	N	3.13	0.39	0.78	0.1	0.2
7o	96	74	CH_2CH_2OH	CH_2CH_3	N	1.56	0.39	1.56	0.2	0.05
Cefdinir						0.1	0.39	0.2	0.1	0.39

N/A: Not applicable.

[a] *S.a.*; *Staphylococcus aureus* 209 P JC-1, *E.c.*; *Escherichia coli* NIHJ JC-2, *K.p.*; *Klebsiella pneumoniae* 12, *P.m.*; *Proteus mirabilis* 1, *P.v.*; *Proteus vulgaris* IAM 1025.

[b] The acetyl substituent is readily cleaved under the MIC measurement conditions.

[c] 5.3:1 mixture of Δ^3 and Δ^2 isomers.

[d] 3.5:1 mixture of Δ^3 and Δ^2 isomers.

[e] 4.6:1 mixture of Δ^3 and Δ^2 isomers.

(conveniently obtained from the corresponding alkynes by lithiation and quench with tributyltin chloride, as outlined in Scheme 2) with the mesylate derivative **1** proceeded in low to moderate yield to provide acetylenes **3a**, **3c**, **3e** and **3f**. For example, treatment of **1** in DMF (~0.5 mmol/ml solution) at 0°C with LiBr (2 equiv.), bis(acetonitrile)palladium(II)chloride (5 mol%) and stannane **2c** (1.1 equiv.) for 1 hour at 0°C and 1 day at room temperature gave **3e** as an off-white powder, after standard extractive work-up and silica-gel chromatography [IR(nujol) 1780 cm^{-1} (β-lactam C=O); ^1H NMR (200 MHz, DMSO-d_6) δ 9.17 (d, 1H, J=9 Hz), 8.15 (s, 1H), 7.54~7.24 (m, 10H), 6.96 (s, 1H), 5.91 (dd, 1H, J=9, 5.1 Hz), 5.22 (d, 1H, J=5.1 Hz), 4.12 (s, 2H), 3.87 and 3.63 (each d, 2H total, AB system, J= 17.9 Hz), 3.18 (s, 3H)]. The lower yields in these reactions were nevertheless acceptable since the more commonly employed triflates were somewhat unstable in our hands. In sharp contrast **1** is exceptionally stable and can be stored for prolonged periods. Whilst we did not establish the identity of reaction by-products in all cases, the Δ^2 isomer of **1** was a major by-product in several cases and could not be induced to undergo coupling with the alkynylstannanes. Desilylation and deformylation steps provided 7-amino cephalosporin derivatives **4a**~**4d** in good yields. For example, **4c** (657.3 mg) was obtained as an off-white powder from **3e** (743 mg) by treatment with concd HCl in MeOH at room temperature for 3.5 hours [^1H NMR (200 MHz, DMSO-d_6) δ 7.51~7.20 (m, 10H), 6.92 (s, 1H), 5.06 (d, 1H, J=5.2 Hz), 4.93~4.85 (m, 1H),

4.10 (s, 2H), 3.80 and 3.53 (each d, 2H total, AB system, $J=17.9$ Hz), 3.17 (s, 3H), 2.41 (br s, 2H)]. These amines were then coupled under standard conditions with the appropriate activated acid derivative **5** and the resulting cephalosporins **6** deprotected by standard methods to provide cephalosporins **7** as free acids or TFA salts. For example, **7i** was produced from the corresponding **6**, containing an oxime *O*-acetate, by treatment with TFA and then with NH_4Cl in MeOH - $NaHCO_3$ (aq) at pH ~ 8 and purification by HP-20 column and lypholization, to give **7i** as an off-white powder [IR (nujol) 1755 cm^{-1} (β-lactam C=O), MS (FAB) 438 (MH^+), ^1H NMR (200 MHz, DMSO-d_6) δ 11.31 (s, 1H), 9.52 (d, 1H, $J=$ 8.1 Hz), 7.14 (br s, 2H), 6.65 (s, 1H), 5.82 (dd, 1H, $J=8.1$, 5 Hz), 5.19 (d, 1H, $J=5$ Hz), 4.27 (s, 2H), 3.77 and 3.49 (each d, 2H total, $J=17.7$ Hz), 3.28 (s, 3H)].

Antibacterial activity for the new cephalosporins prepared in this work is shown in Table 1. MICs were obtained by the standard agar dilution method. For comparison, antibacterial activity of the previously reported alkyne derivatives **7a** and **7b**, as well as cefdinir are included. The effects of a variety of oximino substituents, as well as the effect of thiazole versus thiadiazole were examined. Furthermore, a number of oxygen-containing alkyne substituents were prepared to probe their effect on MIC. As Table 1 clearly indicates, the relatively low activity of **7a** and **7b** against *Staphylococcus aureus* could be significantly improved by introduction of a substituent to the terminal alkyne position; thus **7e**, **7i** and **7k** displayed potent (<1.0 μg/ml) activity. It is clear from the trends shown in Table 1 that only in the case of a free oxime moiety could a major improvement be observed. Activity against various Gram-negative bacteria, especially *Escherichia coli*, was improved when the oxime was alkylated by methyl or ethyl, however in these cases, activity against Gram-positve bacteria was unchanged.

Since **7b**, but not **7a**, was reported to have moderate oral absorbability in the earlier papers,[5,6] we investigated the urinary recovery of the new cephalosporins prepared in this work in rats (PO, 20 mg/kg). However, all compounds showed very low urinary recovery ($<10\%$), this contrasts with cefdinir ($\sim 32.5\%$), **7b** ($\sim 20\%$),[5] and **7a** ($<5\%$).[6]

In summary, we have prepared a series of novel C-3-alkyne-substituted cephalosporins by palladium-catalysed coupling of mesylate **1** and alkynylstannanes, and have shown that in certain cases, antibacterial activity against both Gram-positive and Gram-negative bacteria can be significantly improved, leading to **7i** and **7k** as especially active examples.

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