Pergamon

Tetrahedron Letters, Vol. 38, No. 30, pp. 5261-5264, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01159-3

Efficient Mitomycin C Coupling With Stable p-Nitrophenyl-Benzyl Carbonates Using N-Hydroxybenzotriazole as a Catalytic Additive

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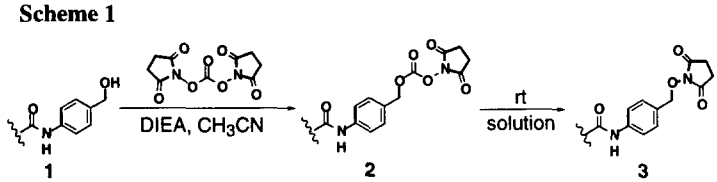
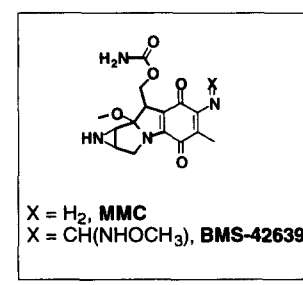
Abstract: Mitomycin C benzyl carbamates were prepared in high yield from stable p-nitrophenyl carbonates in the presence of catalytic amounts of 1-hydroxybenzotriazole/diisopropylethylamine in DMF. © 1997 Elsevier Science Ltd.

Mitomycin C (MMC) is one of the most important anticancer drugs available in the clinic today.[1] Its mechanism of action, DNA crosslinking through bis-alkylation following bioreduction, is dependent on the presence of a free aziridine nitrogen.[2] Prodrugs of MMC, either with metabolically activated small-molecule functionalities[3] or bound to targetable polymers or antibodies,[4] are usually linked through acylation of the aziridine, and are processed enzymatically or by simple hydrolysis. Our interest in targeting MMC and its derivatives through monoclonal antibody-mediated delivery using lysosomally-cleavable peptide linkers required that we develop an efficient method to form MMC carbamates **6** with the peptide benzyl alcohol **1**. The p-aminobenzyloxycarbonyl (PABC) group serves as a self-immolative spacer and is required for proteolytic release of free drug.[5] For our work with doxorubicin (DOX) we were able to use p-nitrophenyl (PNP) carbonates **4** which are very stable and couple easily with the DOX sugar amine.[6] However, MMC, whose aziridine nitrogen is a very sluggish nucleophile, does not react with **4**. More active N-hydroxysuccinimidyl (NHS) carbonates **2** can be prepared,[7] but these react only very slowly with MMC, instead primarily decomposing (ca. 30% at rt in DMF over 18 h) to give benzylated NHS **3** as shown in scheme 1. Chloroformates are normally used to form MMC carbamates but there are two problems that make benzyl chloroformate formation from compounds like **1** difficult: (1) at temperatures above ca. -40°C they decompose to benzyl chlorides, and (2) in the presence of base the monomethoxytrityl (MMT)-amino nitrogen will acylate. In addition, MMC is not very soluble in convenient solvents for chloroformate formation. We found that an efficient way to activate stable PNP-carbonates toward addition by reluctant nitrogen nucleophiles is by *in situ* formation of 1-hydroxybenzotriazole (HOBt) carbonates **5** in DMF in the presence of diisopropylethylamine (DIEA).[8]

Scheme 1





Scheme 2

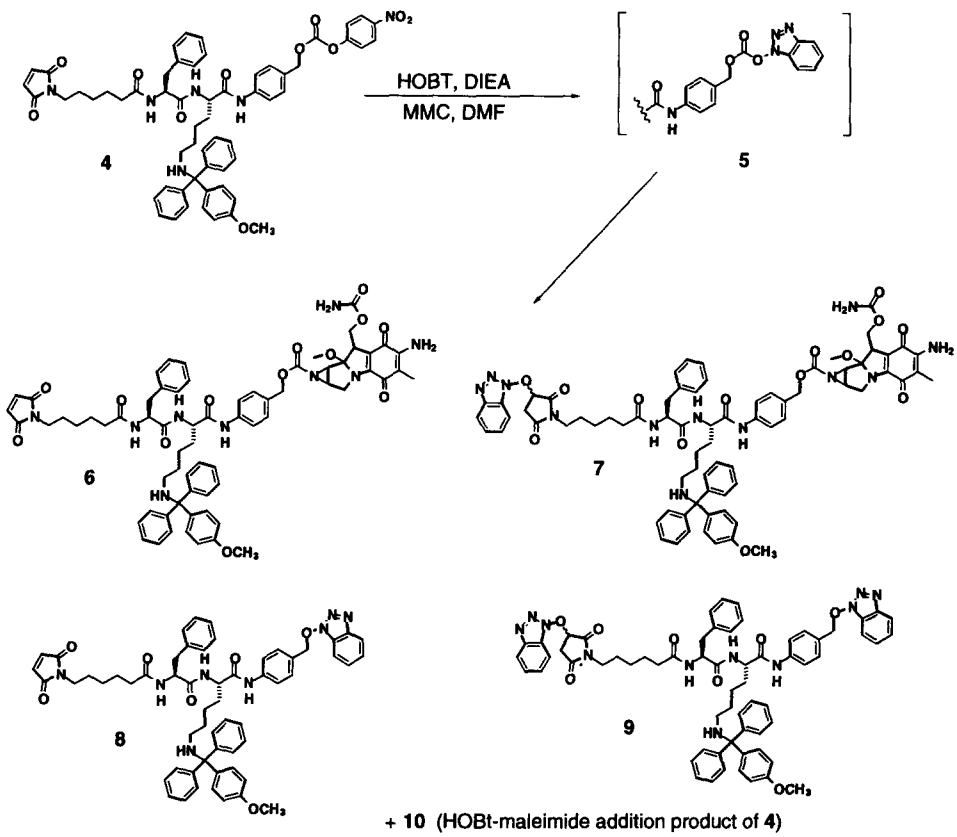


Table 1. Product Formation by HOBt/DIEA Activation of (4) in the Presence of MMC.*

Equiv. HOBt	Equiv. Base	Time, h	(6)	(7)	(8)	(9)	(4)	(10)
10.0	10.0	18	39.4 (33)	45.7	7.6	7.3	0	0
1.1	1.1	18	80.8 (67)	14.8	4.1	0.3	0	0
0.2	1.0	18	75.4	9.3	0	0	14.9	0.4
0.2	1.0	36	83.8	9.1	0	0	6.3	0.8
0.2	1.0	54	89.7 (84)	7.2	0	0	1.9	1.2
0.2	0.2	18	84.1	5.3	0	0	9.9	0.7
0.2	0.2	36	88.0	7.4	0	0	3.8	0.8
0.2	0.2	54	88.9 (83)	8.4	0	0	1.7	1.0

* Values in percent. Yields determined by HPLC except those in parentheses which are isolated yields after silica gel flash chromatography.

Initially we tried an excess of HOBt/DIEA (10 equiv.)[9] reasoning that, since we were trying to form a higher energy carbonate (**5**), this would ensure a reasonable equilibrium. However, as shown in table 1, product yields were low and several side products (**7-9**) were formed.[10] The major product (**7**) resulted from HOBt addition to the maleimide portion of **6**, suggesting a quite high degree of nucleophilicity for the HOBt oxygen. No products from PNP or MMC addition to the maleimide were ever seen. The other products isolated were the alkylated HOBt **8** and its HOBt-maleimide addition product **9** (total yield 15%). The formation of so much alkylated HOBt implied that **5** was formed more readily than we expected and that decomposition was competing with MMC addition. In order to decrease the amount of **5** present *in situ* the amount of HOBt/DIEA was reduced to 1 equiv. This substantially increased the yield of **6** and lowered the amounts of alkylated HOBt side products (**8** and **9**) to <5%. However, HOBt-maleimide addition products (**7** and **9**) still accounted for almost 20% of the product mixture.

When a catalytic amount (0.2 equiv.) of HOBt was used, with either 1 or 0.2 equiv. of DIEA, formation of alkylated HOBt decomposition products was completely suppressed and HOBt-maleimide addition-products were further reduced to 7-9%. Small amounts of the HOBt-maleimide adduct of the starting material **10** were also seen. As expected, the catalytic reactions proceeded more slowly, requiring almost three days to reach satisfactory completion. However, the desired product **6** was much more easily purified and the yields were substantially higher.[11]

Pentafluorophenol (1 equiv.) was also tried as a coupling additive. The reaction proceeded just as cleanly as the catalytic HOBt reactions (no pentafluorophenol-maleimide addition products were detected) but much more slowly: after 54 h 24% of **4** remained unreacted. This presumably reflects a much lower degree of nucleophilicity of the pentafluorophenolate anion in DMF.

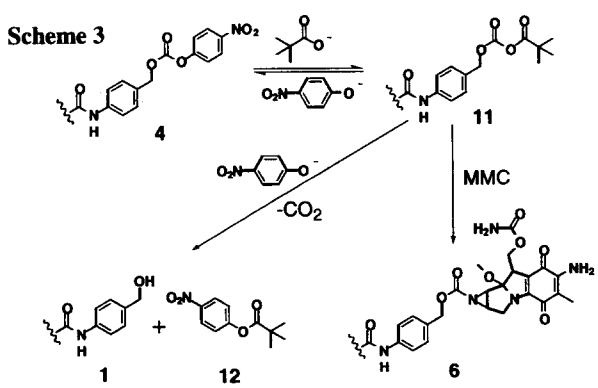
The effect of pivalic acid/DIEA (1 equiv.) as an additive is shown in scheme 3 and table 2. Pivalate was chosen as the carboxyl component least likely to be attacked by a nucleophile due to congestion around the carbonyl carbon. Indeed, MMC only adds to the carbonate carbonyl, as expected. However, p-nitrophenol was able to attack the pivalate carboxyl group giving, after decarboxylation, the benzyl alcohol **1** in a 2:1 ratio over **6** after 72 h with 21% of **4** still remaining, some of it presumably forming from the back reaction shown. When sodium pivalate was used in this reaction only **1** and **12** were formed.

Table 2.
Effect of 1 eq. Pivalate/DIEA on MMC Addition to (4).*

Time, h	(4)	(6)	(12)
18	73.3	8.4	18.3
36	48.0	18.2	33.8
54	36.3	21.8	38.9
72	21.0	26.4	52.6

* Values are in percent. Yields determined by HPLC.

Scheme 3



This methodology has been successfully extended to the more potent MMC derivative BMS-42639 and to other dipeptide linkers. The Lys MMT group was removed[6] from **6** by treatment with chloroacetic acid (10 equiv.)/anisole (100 equiv.) in CH_2Cl_2, and the product coupled to the thiolated BR96 monoclonal antibody.[12] Both immunoconjugates demonstrated excellent, immunospecific activities *in vitro*. Full biological results will be reported in future publications.

In conclusion, we have developed an efficient method to form MMC aziridine carbamates from very stable p-nitrophenylcarbonates using catalytic HOBt/DIEA under mild conditions that largely spare nucleophile-sensitive functionalities such as maleimides as well as MMT-amino groups that can be acylated with chloroformates. This may be a good general method for the preparation of carbamates and carbonates that involve sluggish nitrogen nucleophiles and where chloroformate formation is difficult.

References and Notes

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6. For details see the preceding communication in this issue.
7. Ghosh, A.K.; Duong, T.T.; McKee, S.P. and Thompson, W.J. *Tetrahedron Letters* **1992**, *33*, 2781-2784.
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9. An equivalent amount of base is required since HOBt is acidic enough to remove the MMT protecting group. DIEA has been found to accelerate carbodiimide peptide couplings using HOBt as an additive: Beyermann, M.; Henklein, P.; Klose, A.; Sohr, R. and Bienert, M. *Int. J. Pept. Protein Res.* **1991**, *37*, 252-256.
10. All new compounds gave satisfactory NMR, mass spectral, microanalytical and/or HRMS data.
11. In a typical procedure a mixture of the p-nitrophenylcarbonate **4** (411.7 mg, 0.4 mmol), MMC (146.4 mg, 1.1 equiv.), HOBt (11.3 mg, 0.2 equiv.) and freshly activated 4Å powdered sieves (1 g) in dry DMF (5 mL) were treated with DIEA (0.07 mL, 1 equiv.) and stirred under argon at rt for 54 h. The mixture was filtered, and the filtrate diluted with ethyl acetate (100 mL). The solution was washed with water (4 x 200 mL) and brine, dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica, eluting with 6.25% CH_3OH/CH_2Cl_2, to give the product **6** as a purple solid (407.0 mg, 0.344 mmol, 83%). ^1H NMR ($CDCl_3$) δ 1.10-1.60 (m, 10H), 1.62 and 1.86 (m, each 1H), 1.71 (s, 3H), 2.11 (2xt, 4H), 3.05 (m, 2H), 3.17 (s, 3H), 3.29 (d, 1H), 3.42 (m, 4H), 3.63 (ABq, 1H), 3.73 (s, 3H), 4.26 (t, 1H), 4.42 (d, 2H), 4.71 (q, 1H), 4.91 (m, 1H), 5.00 (ABq, 2H), 5.18 (brs, 1H), 5.43 (br, 1H), 6.31 (d, 1H), 6.62 (s, 2H), 6.77 (d, 2H), 6.89 (d, 1H), 7.10-7.55 (m, 17H), 8.80 (brs, 1H). MS: (FAB) 1225.4 $(MH)^+$, 1263.5 $(M+K)^+$. HRMS: Calc. for $C_{68}H_{74}N_9O_{13}$: 1224.5406. Found: 1224.5385.
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(*Received in USA* 6 *May* 1997; *accepted* 9 *June* 1997)