General and Efficient Method for the Solution- and Solid-Phase Synthesis of Vancomycin Carboxamide Derivatives

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Vancomycin (**1**, Figure 1) prototypifies the glycopeptide family of antibiotics and represents the treatment of choice for infections due to methicillin-resistant *Staphylococcus aureus*. Vancomycin and its congeners exhibit a novel receptor-like mode of action—they bind to peptidoglycan precursors and prevent their incorporation into the polymeric bacterial cell wall.[1] We have initiated a research program directed at the design and preparation of vancomycin derivatives that not only bind to peptidoglycan precursors but also catalyze their decomposition. The long-term goal of this work is to determine whether "catalytic antibiotics" display enhanced potency relative to stoichiometrically acting counterparts. Recently we reported a solution-phase method for synthesis of vancomycin carboxamide derivatives via dicyclohexylcarbodiimide-mediated coupling of vancomycin with primary amines.[2] This methodology allowed the preparation of a number of derivatives and the discovery of unprecedented carbamate hydrolase activity by these compounds, but coupling rates were slow and isolated yields were often low. This led us to search for an improved coupling protocol. We now report that use of 2-(1-hydroxybenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)[3] affords rapid and efficient coupling of vancomycin with primary amines in solution and that this method can be extended to the preparation of vancomycin peptide conjugates by solid phase methods.

The preparation of vancomycin benzylamide illustrates the improved solution-phase coupling protocol. Vancomycin hydrochloride (100 mg, 67 μmol) was dissolved in 1 mL of dry dimethyl sulfoxide (DMSO). To this was added 1 mL of dry dimethylformamide (DMF) and 14.4 mg (2 equiv) of benzylamine hydrochloride. The mixture was cooled to 0 °C, and 0.22 μL (1.5 equiv) of 0.45 M HBTU solution in DMF was added,[4] followed by 58 μL (5.0 equiv) of diisopropylethylamine (DIEA). The reaction was then allowed to warm to room temperature. After 4 h, analytical reversed-phase HPLC showed near-complete loss of vancomycin and appearance of a less polar product. The product was purified by preparative reversed-phase HPLC and lyophilized to afford 95 mg (55 μmol, 82%) of vancomycin benzylamide **4** as the bis-(trifluoroacetate) salt. The structure of this and all vancomycin derivatives was established using 400 MHz ^{1}H NMR and FAB-MS. ^{1}H NMR showed resonances attributable to vancomycin as well as a new triplet at δ 8.29 (amide N*H*), a multiplet at δ 7.30 (phenyl C*H*s), and a singlet at δ 4.45 (PhCH_2). The FAB-MS exhibited an ion at *m/z* 1538.4 consistent with the molecular weight calculated for the parent ion (MH^+) $C_{73}H_{84}N_{10}O_{23}Cl_2$, 1538.5. Also observed were fragment ions at *m/z* 1503.4, 1394.2, and 1234.2, which correspond to loss of methylamine, vancosamine, and vancosamine plus glucose, respectively.[5]

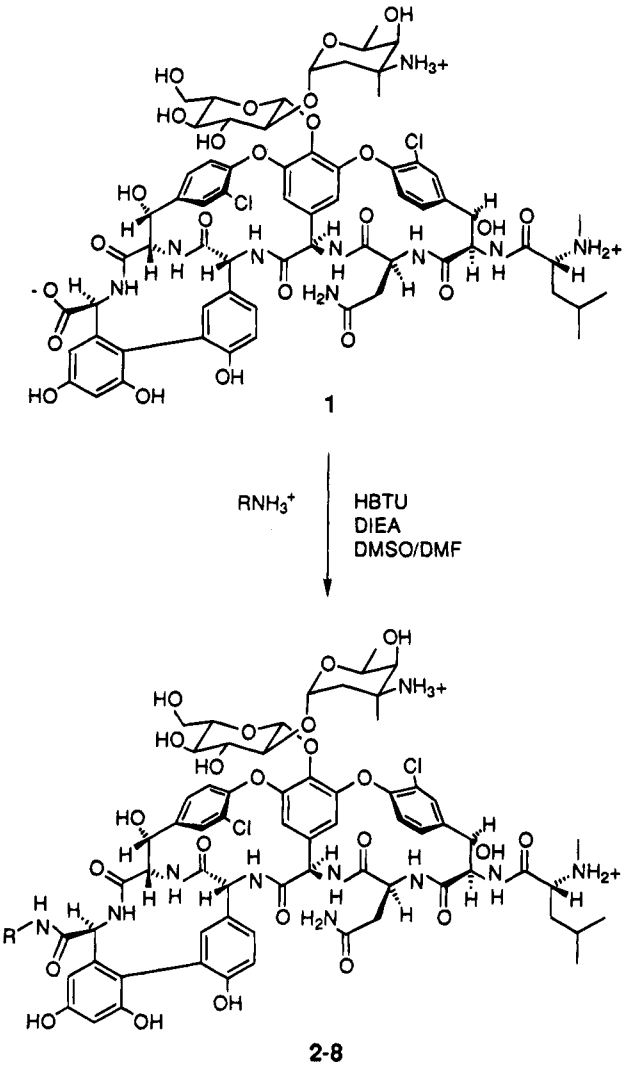

Figure 1. General method for synthesis of vancomycin carboxamide derivatives.

Table 1 lists vancomycin carboxamide derivatives **2–8** prepared by HBTU-mediated coupling with primary alkylamines (≤3 equiv) bearing aryl, hydroxy, ester, amine, and disulfide functional groups. The isolated yields of these compounds ranged from 55% to 82% after reaction times of ≤8 h. Hydrophobic amines, such as benzylamine and 2-phenylethylamine, reacted more quickly and completely with vancomycin than did some of the more hydrophilic amines, such as the diamines. Relative to reaction with primary amine partners, intermolecular self-coupling of vancomycin with its hindered secondary or neopentylic primary amines was not found to be a significant competing process. Two limitations to the HBTU-mediated coupling protocol have been discovered to date. First, coupling reactions involving aromatic and secondary amines (aniline and diethy-

(1) Perkins, H. R.; Nieto, M. *Pure Appl. Chem.* **1973**, *35*, 371–381.
(2) Shi, Z.; Griffin, J. H. *J. Am. Chem. Soc.* **1993**, *115*, 6482–6486.
(3) (a) Dourtoglou, V.; Ziegler, J.; Gross, B. *Tetrahedron Lett.* **1978**, 1269–1272. (b) Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C. *Synthesis* **1984**, 572–574. (c) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *20*, 1927–1930.
(4) HBTU was obtained from Applied Biosystems, Inc., in the form of the FastMoc kit. The solution of HBTU also contains 0.45 M hydroxybenzotriazole (HOBT).
(5) (a) Nagarajan, R.; Schabel, A. A.; Occolowitz, J. L.; Counter, F. T.; Ott, J. L. *J. Antibiot.* **1988**, *41*, 1430–1438. (b) Nagarajan, R.; Schabel, A. A.; Occolowitz, J. L.; Counter, F. T.; Ott, J. L.; Felty-Duckworth, A. M. *J. Antibiot.* **1989**, *42*, 63–72.

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Table 1. Synthesis of Vancomycin Carboxamide Derivatives

compd	R	reaction time (h)	isolated yield (%)	FAB-MS, *m/z* (calculated for MH^+)	FAB-MS, *m/z* (obsd)
2	$CH_3CH_2CH_2-$	3	78	1490.5 ($C_{69}H_{83}N_{10}O_{23}Cl_2$)	1490.4
3	$HOCH_2CH_2-$	7	80	1492.5 ($C_{68}H_{81}N_{10}O_{24}Cl_2$)	1492.3
4	$PhCH_2-$	4	82	1538.5 ($C_{73}H_{83}N_{10}O_{23}Cl_2$)	1538.4
5	$PhCH_2CH_2-$	4	82	1552.5 ($C_{74}H_{85}N_{10}O_{23}Cl_2$)	1551.4
6	$MeOCOCH_2CH_2CH_2-$	6	57	1548.5 ($C_{71}H_{85}N_{10}O_{25}Cl_2$)	1547.7
7	$H_2NCH_2CH_2NHCH_2CH_2NHCH_2CH_2-$	8	55	1577.6 ($C_{72}H_{92}N_{13}O_{23}Cl_2$)	1577.6
8	$H_2NCH_2CH_2SSCH_2CH_2-$	6	72	1583.5 ($C_{70}H_{86}N_{11}O_{23}Cl_2S_2$)	1583.4
9	CH_3CH_2O-Gly-Gly-		95	1591.5 ($C_{72}H_{86}N_{11}O_{26}Cl_2$)	1591.5
10	HO-Gly-Gly-		25	1563.5 ($C_{70}H_{82}N_{11}O_{26}Cl_2$)	1563.4
11	HO-D-Ala-D-Ala-L-Lys(Ac)-GABA-$(Gly)_5$-		20	2134.8 ($C_{93}H_{121}N_{19}O_{35}Cl_2$)	2132.2
12	HO-L-Ala-L-Ala-		34	1590.5 ($C_{72}H_{86}N_{11}O_{26}Cl_2$)	1590.4
13	HO-D-Glu-L-His-L-Lys-		20	1841.6 ($C_{83}H_{101}N_{15}O_{29}Cl_2$)	1841.7

lamine, respectively) produced a complex mixture of products from which the desired carboxamide derivatives could not be isolated. Second, free thiol groups interfere with the coupling process.

The vancomycin–peptide ester conjugate Van-Gly-Gly-OEt (**9**) was synthesized in solution in high yield by coupling 1.5 equiv of vancomycin hydrochloride with glycylglycine ethyl ester hydrochloride. The free acid Van-Gly-Gly-OH (**10**) was isolated in 75% yield after stirring **9** with 2.0 equiv of lithium hydroxide in 1:6 H_2O:DMF (pH 8.5) for 12 h. The efficacy of the HBTU-mediated coupling protocol in this instance suggested that it could be extended to the preparation of vancomycin derivatives by solid phase methods.[3c] To test this hypothesis, FMOC-protected glycylglycine was synthesized on 0.25 mmol scale on super acid sensitive resin[6] by standard manual stepwise solid phase methods. The FMOC group was removed with 20% piperidine, and 557 mg of vancomycin (0.375 mmol, 1.5 equiv), dissolved in 5 mL of DMSO, was added to the resin. This was followed by 0.86 mL (1.5 equiv) of 0.45 M HBTU solution in DMF and 0.34 mL (5 equiv) of DIEA. The reaction was agitated for 12 h in a wrist shaker, the resin was washed several times with *N*-methylpyrrolidone (NMP), and the coupling reaction was repeated. The resin was washed with NMP followed by dichloromethane (DCM) and then cleaved with 2% trifluoroacetic acid in DCM for 20 min at room temperature. After the supernatant-containing uncoupled dipeptide was removed, the resin was treated with DMSO to extract the vancomycin–peptide conjugate. Evaporation of DMSO afforded 110 mg (0.063 mmol, 25%) of **10**. This material was homogeneous by reversed-phase HPLC, and its ^1H NMR and FAB-MS matched those of the product synthesized by solution phase methods. In a more complex example, the nonapeptide conjugate Van-$(Gly)_5$-γ-aminobutyric acid-L-Lys(Ac)-D-Ala-D-Ala-OH (**11**) was isolated in 20% yield after reversed-phase HPLC purification of the crude product extracted from the resin with DMSO. Also prepared by solid-phase synthesis were Van-L-Ala-L-Ala-OH (**12**) and Van-L-Lys(Boc)-L-His(Trt)-D-Glu(OtBu)-OH, which demonstrate the ability of HBTU to mediate the coupling between vancomycin and resin-bound peptides containing *N*-terminal α-substituted amino acids. The last conjugate also allowed a preliminary test of the stability of vancomycin's glycosidic linkages under the acidic conditions needed to remove representative sidechain protecting groups. The crude peptide was treated at a concentration of 5 mg/mL with 5% TFA in DCM and stirred at 4 °C for 6.5 h. After purification by HPLC, Van-L-Lys-L-His-D-Glu-OH (**13**) was obtained in 20% overall yield. Significant amounts of the corresponding deglycosylated conjugates were not observed.

The HBTU-mediated coupling protocol greatly increases the efficiency with which vancomycin derivatives may be prepared in solution and the range of conjugates which may be obtained. Extension of this methodology to support-bound amine coupling partners represents, to our knowledge, the first method for solid-phase semisynthetic modification of a glycopeptide antibacterial agent. We intend to apply this methodology to the preparation of diverse libraries of glycopeptide derivatives by combinatorial approaches[7] and to screen these libraries for enhanced binding to peptidoglycan precursors (including those from vancomycin-resistant bacteria[8]) and antibacterial activities.

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Supplementary Material Available: Copies of ^1H NMR spectra for vancomycin carboxamide derivatives (12 pages).

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(6) (a) Mergler, M.; Tanner, R.; Gosteli, J.; Grogg, P. *Tetrahedron Lett.* **1988**, *29*, 4005–4008. (b) Mergler, M.; Nyfeler, R.; Tanner, R.; Gosteli, J.; Grogg, P. *Tetrahedron Lett.* **1988**, *29*, 4009–4012.

(7) For recent reviews see: (a) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1385–1401. (b) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385–1401.

(8) (a) Courvalin, P. *Antimicrob. Agents Chemother.* **1990**, *34*, 2291–2296. (b) Billot-Klein, D.; Gutmann, L.; Collatz, E.; van Heijenoort, J. *Antimicrob. Agents Chemother.* **1992**, *36*, 1487–1490. (c) Wright, G. D.; Walsh, C. T. *Acc. Chem. Res.* **1992**, *25*, 468–473. (d) Handwerger, S.; Pucci, M. J.; Volk, K. J.; Liu, J.; Lee, M. S. *J. Bacteriol.* **1992**, *174*, 5982–5984.