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THE SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 2-CARBOLINYL-CARBAPENEMS: POTENT ANTI-MRSA/MRCNS AGENTS

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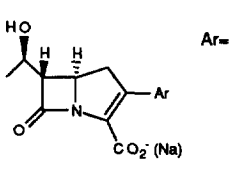
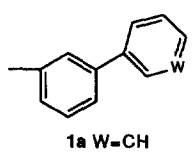
Abstract. A series of 2-carbolinyl-carbapenems was prepared *via* the Stille stannane coupling reaction. This new class of antibiotics exhibited potent activity *in vitro* against methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase negative staphylococci (MRCNS) as well as a broad spectrum of antibacterial activity. A high resistance to the mammalian dehydropeptidase, DHP-1, was also observed.

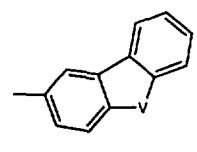
Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase negative staphylococci (MRCNS) infections have increased dramatically in recent years.[1] A program in these laboratories to investigate novel 2-aryl carbapenems with increased chemical and metabolic stability identified the *meta*-biphenyl pharmacophore of **1a**[2] as desirable for potent activity against gram-positive organisms including MRSA. Appending a 3-pyridyl moiety at the *meta* position of the phenyl ring as in **1b**[2] also achieved potent activity. Conformationally restricting the phenyl rings with a central five-membered ring connected through a heteroatom or with a carbonyl moiety provided various planar tricyclic structures **2** with increased anti-MRSA/MRCNS activity.[3] To determine the cumulative effects of the *meta*-3-pyridyl ring of **1b** and the planar tricyclic structure of **2**, a series of 2-carbolinyl carbapenems (azacarbazoles) **3** were prepared with a nitrogen bridging the *meta*-biphenyl rings. The nitrogen of the pyridyl ring of **3** was introduced into all the four nonbridging positions to give the α, β, γ, and δ carbolinyl derivatives **3a-d** and the quaternary analogs, **3e-i**, were also prepared. Two α-carbolinyl analogs with the carboline attached at the 5-carbon, **4a**, and the 7-carbon, **4b**, were also prepared as well as the 9-des-methyl quaternary β-carbolinyl derivative **5**. The above compounds were evaluated for antimicrobial activity in a primary antibacterial screen and the structure-activity relationships are presented. Of particular interest were the 1,9-dimethyl-α-carbolinyl-, **3e**, the 2,9-dimethyl-β-carbolinyl-, **3f**, and the 2-methyl-β-carbolinyl-carbapenem, **5**, which all displayed excellent anti-MRSA/MRCNS activity as well as demonstrating a broad spectrum of antibacterial activity.



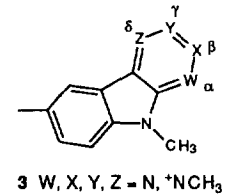
Ar=



1a W=CH
b W=N

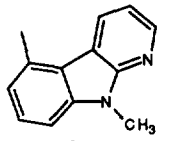


2 V=O,S,CO

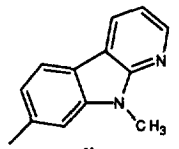


3 W, X, Y, Z = N, $^+NCH_3$ or CH

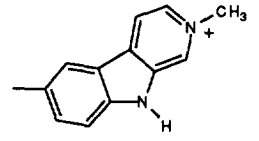
a W=N	**e** W=$^+NCH_3$
b X=N	**f** X=$^+NCH_3$
c Y=N	**g** Y=$^+NCH_3$
d Z=N	**h** Z=$^+NCH_3$
	i W=$^+NCH_2CH_3$



4a



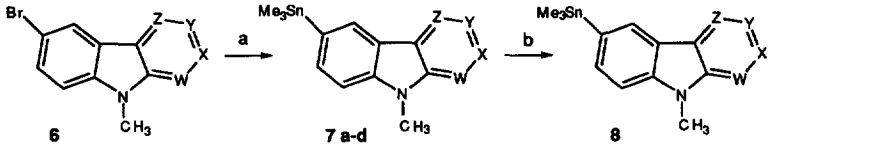
4b



5

Chemistry. The synthetic approach utilized to prepare the 2-carbolinyl-carbapenems was based on the modified Stille stannane coupling reaction reported previously.[4] In Scheme 1 the requisite carbolinyl bromides **6a-d**,[5] were converted to the aryl trimethylstannanes **7a-d** with hexamethylditin (1.2 equiv.), tetrakistriphenylphosphinepalladium (0) (5 mol%) and triphenylphosphine (3 mol%) in refluxing toluene. The quaternized carbolinyl stannanes **8e-i** were subsequently prepared by alkylation of **7a-d** with methyl or ethyl triflate.

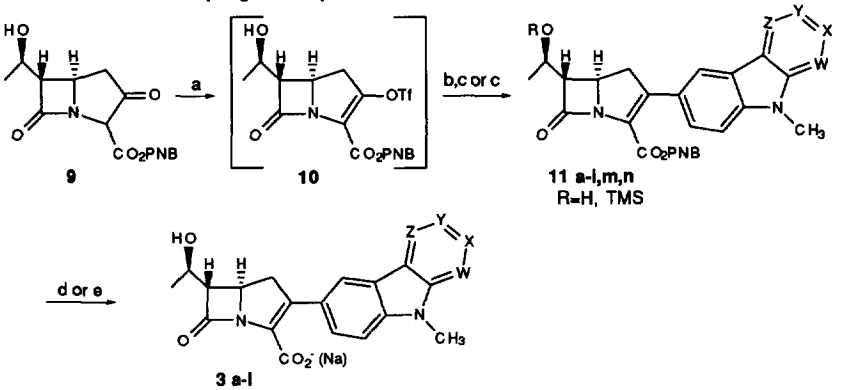
SCHEME 1. Preparation of Aryl Stannanes.



a.) $(CH_3)_6Sn_2$, $Pd(PPh_3)_4$, PPh_3, $PhCH_3$, 110°C b.) MeOTf or EtOTf, CH_2Cl_2, 0°C

The general method to append the 2-carbolinyl moiety to the carbapenem involved the palladium-catalyzed coupling of the aryl stannanes to the activated carbapenem as shown in Scheme 2. The enol triflate **10** was formed *in situ* from the bicyclic ketoester **9** using the conditions described by Rano *et al.*[4] The aryl stannanes were added to **10** with N-methylpyrolidinone followed by Pd_2·dba_3·$CHCl_3$ (2 mol%) and diisopropylamine hydrochloride. The reaction was warmed immediately to room temperature and then aged from thirty minutes to three hours. In examples employing the unprotected C8 hydroxyl moiety (**11**, R=H), the products were isolated by precipitation from dichloromethane in yields of 30-60%. Preferably, the hydroxyl was protected with a trimethylsilyl moiety (**11**, R=TMS) by treating **10** with TMS triflate and triethylamine. The TMS protected adduct **11** allowed for a more facile purification by silica gel chromatography and therefore higher yields (50-87%). The TMS moiety was removed with HCl prior to hydrogenation of the *p*-nitrobenzyl ester with 10% palladium-on-carbon in aqueous THF. Subsequent purification on either reverse phase preparatory plates or a Lobar RP18 HPLC column provided the carbapenems **3a-i** in yields ranging from 13-62%.

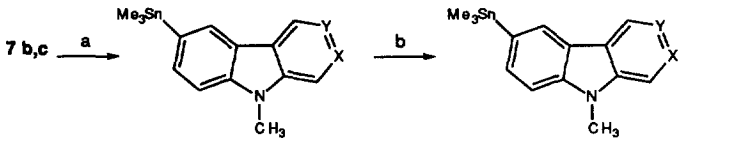
SCHEME 2. Cross Coupling and Deprotection.



a.) DIPEA, Tf_2O, THF, -70°C b.) if R=TMS, Et_3N, TMS-triflate, -70°C c.) aryl stannanes **7a,d**, **8e-i** or **13m,n**, NMP, $Pd_2(dba)_3CHCl_3$, $[(CH_3)_2CH]_2NH$·HCl, -70°C to RT d.) If R=TMS, 1. HCl, 2. Pd/C, H_2,THF-H_2O e.) if R =H, Pd/C, H_2,THF-H_2O ($NaHCO_3$ with neutral species).

The cross-coupling of the α- and δ- carbolinyl stannanes **7a,d** proceeded smoothly; however, coupling of the β- and the γ-carbolinyl stannanes **7b,c** failed to provide the desired adducts, presumably due to the complexation of the catalyst with the more accessible lone pairs on the pyridyl nitrogens. This problem was circumvented by first protecting the nitrogens with a methoxy moiety[6] which was concomitantly cleaved during the hydrogenation of the *p*-nitrobenzyl ester. Thus, as shown in Scheme 3, the β- and γ-carbolinyl stannanes **7b,c** were first converted to the N-oxides **12j,k** with *m*-chloroperoxybenzoic acid and subsequently alkylated with methyl triflate to provide the requisite N-methoxy protected β- and γ-carbolinyl stannanes **13m,n**.

SCHEME 3. Preparation of N-methoxy Protected Aryl Stannanes.



12 j X=NO, Y=CH
k Y=NO, X=CH

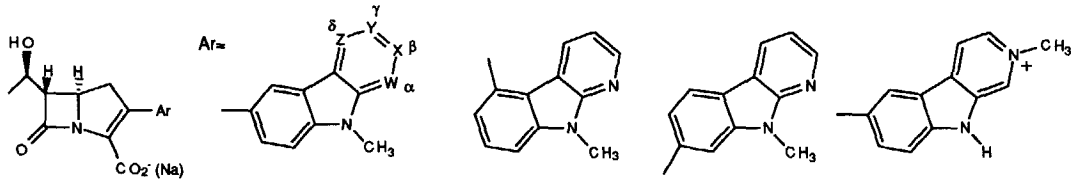
13 m X=$^{+}$N-OCH$_3$ $^{-}$OTf, Y=CH
n Y=$^{+}$N-OCH$_3$ $^{-}$OTf, X=CH

a.) MCPBA, CH_2Cl_2, $NaHCO_3$, 0°C b.) MeOTf, CH_2Cl_2 0°C

The α-carbolinyl-carbapenems **4a,b** were prepared in the same manner as **3a,d** from the 5-bromo and 7-bromo-9-methyl-α-carbolines.[7] The 9-des-methyl-β-carbolinyl-carbapenem **5** was prepared by treating 6-bromo-β-carboline[5b] with acetic anhydride and pyridine at ambient temperature to provide 6-bromo-9-acetyl-β-carboline. Conversion to the trimethylstannyl synthon, quaternization of the β-nitrogen with methyl triflate and coupling proceeded as previously described in Schemes 1 and 2. During the hydrogenation of the *p*-nitrobenzyl ester adduct the 9-acetyl moiety was concomitantly cleaved to provide **5**.

Biology. The 2-carbolinyl-carbapenems were evaluated for antibacterial activity relative to imipenem as shown in Table 1. In the neutral carbolinyl series, the β-carbolinyl analog **3b** was about two fold more potent than the α- and γ- carbolinyl analogs **3a** and **3c** against MRSA (relative potencies of 21, 8, and 11 for **3b**, **3a**, and **3c**, respectively). The δ-carbolinyl analog **3d** and the 7-α-carbolinyl analog **4b** were the least potent with relative activities to imipenem of less than six. In this neutral series the β-carbolinyl analog **3b** also demonstrated the greatest potency vs MRCNS while the γ-carbolinyl derivative **3c** was the most active against *Proteus*. In all of the above examples, quaternization of the carbolinyl pyridyl nitrogen enhanced anti-MRSA activity. This effect was most pronounced in the α-carbolinyl series as seen by the increase in relative potency from 8 for the 9-methyl-α-carbolinyl analog **3a** to 30 for the 1,9-dimethyl-α-carbolinyl analog **3e** and 21 for the 1-ethyl-9-methyl-α-carbolinyl analog **3i**. The 9-des-β-carbolinyl analog **5** was slightly more active than the 2,9-dimethyl-β-carbolinyl compound **3f** vs MRSA, but only half as active against MRCNS. Noteworthy was the dramatic increase in antibacterial activity against MRCNS for the dialkyl α-carbolinyl carbapenems **3e** and **3i**, and the dimethyl-β analog **3f**, with relative potencies of 313, 277 and 287, respectively. A two to three-fold increase in potency against *E. coli*, *Enterobacter*, and *Serratia* was observed for **5** vs the dialkyl analog **3f**. Quaterization was the least effective in the γ-carbolinyl-series (**3c** vs **3g**); MRCNS activity remained constant while *Serratia* and *Proteus* activity decreased approximately two-fold. Useful broad spectrum antibacterial activity was observed for **3e,f,i** and **5** compared to imipenem against all the organisms tested except *Ps. aeruginosa* and

Table 1. Antibacterial Activity *in Vitro* and DHP-I Stability of 2-Carbolinyl-Carbapenems.



3 W, X, Y, Z = N, $^+NCH_3$ or CH

a W=N e W=$^+NCH_3$
b X=N f X=$^+NCH_3$
c Y=N g Y=+NCH3
d Z=N h Z=$^+NCH_3$
l W=$^+NCH_2CH_3$

Species (No.)[a]	MIC[b], μg/mL Imipenem	Fold Improvement in Activity Relative to Imipenem[c]											
		3a	3b	3c	3d	3e	3f	3g	3h	3i	4a	4b	5
MRSA[d] (1)	34 - 45	8.0	21	11	5.3	30	23	14	12	21	13	4.9	28
MRCNS[e] (1)	67 - 73	8.6	49	40	4.5	313	287	40	27	277	12	4.1	166
MSSA[f] (4)	0.009 - 0.018	0.17	0.29	0.56	0.20	0.49	0.38	0.59	0.58	0.43	0.26	0.15	0.42
Enterococcus spp. (3)	2.8 - 4.3	2.1	2.3	4.0	1.2	3.9	3.8	3.7	2.5	3.7	1.4	1.5	2.8
E. coli (5)	0.68 - 0.89	0.12	0.35	1.8	0.31	4.8	3.8	1.5	1.3	2.8	0.25	0.15	9.2
Enterobacter spp. (6)	0.49 - 0.62	0.034	0.14	0.98	0.065	2.8	2.5	0.94	1.2	1.4	0.092	0.041	7.7
Klebsiella spp. (5)	0.59 - 1	0.059	0.17	0.94	0.16	3.0	2.5	1.2	1.1	1.4	0.08	0.054	2.8
Serratia spp. (2)	0.76 - 1.5	0.10	0.20	4.8	0.37	4.5	3.6	2.1	2.2	3.2	0.38	0.051	12
Proteus spp. (5)	0.95 - 1.5	1.4	3.8	20	6.6	26	21	14	9.3	11	1.2	0.59	25
Ps. aeruginosa (5)	0.31 - 0.44	0.007	0.008	0.018	0.006	0.057	0.035	0.026	0.018	0.035	0.009	0.007	0.027
		DHP-I Susceptibility Relative to Imipenem[g]											
	Imipenem	3a	3b	3c	3d	3e	3f	3g	3h	3i	4a	4b	5
DHP-I susceptibility	(1.0)	0.06	0.034	0.00	1.8	0.05	0.01	0.007	0.00	0.01	0.000	0.02	0.010

a. Agar disc diffusion assay method (ref. 9). In the instances where more than one strain per species was tested, a geometric mean of the MICs (referred to as a species index) was calculated for each species. b. Range of imipenem species indices achieved from several tests given as a reference. c. Relative potency, based on species indices for an individual test, is calculated by dividing the species index of imipenem by the species index of test compound. d. Methicillin-resistant *S. aureus*. e. Methicillin-resistant coagulase negative staphylococci. f. Methicillin-susceptible *S. aureus*. g. DHP-I (porcine) susceptibility is given as subject compound hydrolysis rate divided by hydrolysis rate with imipenem as a substrate (ref. 10).

methicillin-susceptible *S. aureus* (MSSA). All of the carbapenems tested except **3d** exhibited a high resistance to the mammalian dehydropeptidase, DHP-1, which is typical for 2-aryl carbapenems.[8]

Several of the above compounds were selected for further evaluation *in vitro* against an expanded panel of MRSA and MRCNS strains, the majority of which are imipenem resistant. The carbolinyl-carbapenems tested were compared to both vancomycin and imipenem as shown in Table 2 and all were substantially more active *in vitro* than imipenem against MRSA and MRCNS. The quaternary α-carbolinyl **3e** was two-fold more potent vs MRSA and four-fold more active against MRCNS than the neutral analog **3a**. Compound **4a** with the carboline attached at the 5-carbon, showed similar activity against MRSA and MRCNS when compared with **3a**. In the β-carbolinyl series the MRSA activity was similiar for **3b** and **3f** while the MRCNS potency improved two-fold for the quaternary analog **3f**. The γ-analogs **3c** and **3g** exhibited identical activity vs MRSA and MRCNS. The quaternary α- and β-analogs **3e**, **3f** and **5** were two-fold more potent than the γ- and δ-analogs **3g** and **3h** against MRSA and four-fold more potent vs MRCNS. The MRSA *in vitro* activity of the most potent analogs **3e**, **3f** and **5** was one-half that of vancomycin. Against MRCNS *in vitro*, **3f** and **5** were equipotent to vancomycin whereas **3e** exhibited a two-fold increase in potency vs vancomycin.

Table 2. *In Vitro* MRSA/MRCNS Activity of 2-Carbolinyl-Carbapenems.

	MIC, μg/mL[a]						MRSA/MRCNS Activity Relative to Standard Antibiotics [b]	
	MRSA (N=13)			MRCNS (N=9)				
Compound	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Imipenem	Vancomycin
3a	1 - 16	4	8	4 - 32	8	16	16x / >8x	0.25x / 0.25x
3b	0.5 - 4	2	4	1 - 8	4	8	>32x / >16x	0.5x / 0.5x
3c	0.5 - 8	4	8	2 - 16	8	16	16x / 16x	0.25x / 0.25x
3e	0.5 - 4	2	4	1 - 4	4	4	32x / >32x	0.5x / 2x
3f	1 - 8	2	4	1 - 4	4	4	32x / 32x	0.5x / 1x
3g	1 - 8	4	8	2 - 16	8	16	16x / >8x	0.25x / 0.5x
3h	1 - 8	4	8	2 - 16	8	16	16x / >8x	0.25x / 0.5x
4a	1 - 16	4	8	2 - 16	8	16	>16x / >8x	0.25x / 0.25x
5	0.5 - 8	2	4	1 - 4	4	4	32x / >32x	0.5x / 1x

a. Broth microtube dilution method (ref. 11). Mueller-Hinton Broth + 2% NaCl, inoculum ~10^5 CFU/mL, incubation at 35°C for 48 hr. b. Relative activity based on MIC_{90} values for individual tests. Imipenem MIC_{90}s ranged from 128 - >128 μg/mL for MRSA and MRCNS; Vancomycin MIC_{90}s were 2 μg/mL for MRSA and ranged from 4-8 μg/mL for MRCNS.

Conclusion. Quaternization of the pyridyl nitrogen of a series of 2-carbolinyl-carbapenems was found to enhance antibacterial activity compared to the neutral species. This effect was most pronounced in the α- and β-series vs MRSA and especially against MRCNS. Thus, the favored positions for the pyridyl nitrogen was either the α- or β- positions in the quaternary series, whereas the β- and γ- positions provided more active compounds in the neutral series. The most interesting compounds described were the quaternary α- and β–carbolinyl analogs **3e, f** and **5** which demonstrated a broad spectrum of antibacterial activity as well as potent *in vitro* MRSA and MRCNS activity.

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6. Stephenson, L.; Warburton, W. K. *J. Chem. Soc. (C)*, **1970**, 1355.

7. The 5-bromo and 7-bromo-9-methyl-α-carbolines were prepared by the method in ref. 4a but utilizing 3-bromo-N-methylaniline and 2-chloro-3-nitropyridine as the starting materials. During the diazotization and concomitant ring closure with copper powder both the 5-bromo-9-methyl-α-carboline (21%) and 7-bromo-9-methyl-α-carboline (14%) were obtained.

8. (a.) Cama, L. D.; Wildonger, K. J.; Guthikonda, R.; Ratcliffe, R. W.; Christensen, B. G. *Tetrahedron*, **1983**, *39*, 2531. (b.) DiNinno, F.; Muthard, D. A.; Salzmann, T. N. *BioMed. Chem. Lett.*, **1993**, *3*, 2187.

9. (a.) The antibacterial activity of the synthetic carbapenems was determined by a disc diffusion assay using imipenem as the internal standard. Inhibitory concentration at the edge of the zone of inhibition was computed for each compound and for imipenem by a rearrangement of equation 3 in ref. 9b, which takes into account the differing molecular weights and resultant diffusion constants for each compound. Strains were individually calibrated for their critical times. The ratio of inhibitory concentration to that of imipenem is stated in the tables. For comparison, the range of MICs for imipenem are shown for the strains employed. (b.) Humphrey, J. H.; Lightbrown, J. W. *J. Gen. Microbiology*, **1952**, *7*, 129.

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