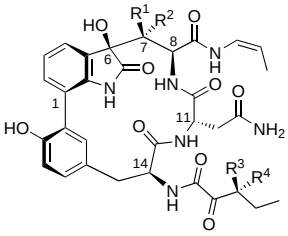
Synthesis of the Functionalized Macrocyclic Core of Proteasome Inhibitors TMC-95A and B**

Songnian Lin and Samuel J. Danishefsky*

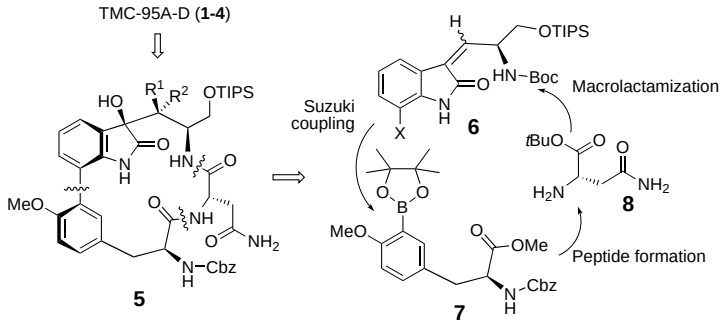
TMC-95A (**1**) and its diastereomers, TMC-95B, C, and D (**2**–**4**,), are cyclic peptides recently isolated as fermentation products of *Apiospora montagnei* Sacc. TC1093 derived from



TMC-95	R^1	R^2	R^3	R^4
A (**1**)	H	OH	CH_3	H
B (**2**)	H	OH	H	CH_3
C (**3**)	OH	H	CH_3	H
D (**4**)	OH	H	H	CH_3

soil samples.[1, 2] Biological studies[1] have shown that TMC-95A inhibited the chymotrypsin-like (ChT-L), trypsin-like (T-L), and peptidoglutamyl-hydrolyzing (PGPH) activities of the 20S proteasome[3, 4] with IC_{50} values of 5.4, 200, and 60 nM, respectively. TMC-95B inhibited these activities to the same extent as TMC-95A, while TMC-95C and D were 20 to 150 times weaker. TMC-95A has also shown cytotoxic activities against HCT-116 (human colon carcinoma cells) and HL-60 (human promyelocytic leukemia cells) with IC_{50} values of 4.4 and 9.8 μM, respectively. These four diastereomers are structurally characterized as novel cyclic peptides containing L-tyrosine, L-asparagine, highly oxidized L-tryptophan, (*Z*)-1-propenylamine, and 3-methyl-2-oxopentanoic acid moieties. Although the phenyl-indole ring attachment is found in a few natural products such as chloropeptin,[5] complestatin,[6] diazonamide,[7] and the kistamicins,[8] the presence of an oxindole ring in such macrocyclic systems is apparently rare. The structural novelty of the TMC compounds and the biological issues they raise prompted us to embark on a program directed to their total synthesis.

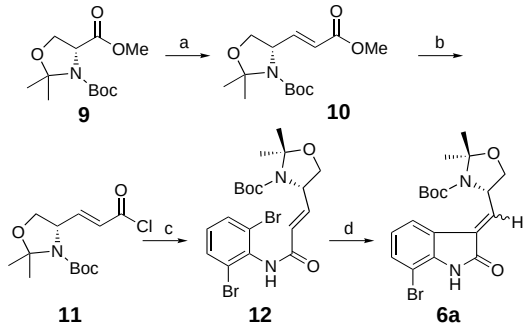
We envisioned that the 17-membered ring could be fashioned by macrolactamization. With the ring in place, attention could then be directed to inclusion of the unusual (*Z*)-1-propenylamide and 3-methyl-2-oxopentanoate side chains at C-8 and C-14, respectively. The installation of hydroxy groups at C-6 and C-7 could in principle be conducted prior to, or after, macrocyclization (Scheme 1). We



Scheme 1. Retrosynthetic plan. TIPS = triisoproylsilyl, Cbz = benzoxycarbonyl, Boc = *tert*-butoxycarbonyl, X = Br or I.

further foresaw that a Suzuki reaction,[9] which would allow the joining of **6** and **7**, might be employed to reach macrolactamization precursors en route to **5**. Below, we report the encouraging realization of several important milestones pursuant to this rough blueprint.

The synthesis of oxindole **6a** was first attempted by using a proposed palladium-mediated intramolecular Heck reaction of substituted *N*-acryl-2,6-dibromoaniline **12** (Scheme 2).



Scheme 2. Attempted synthesis of 7-bromooxindole **6a**. a) 1. DIBAL/toluene, −78 °C, 1 h; 2. methyl (triphenylphosphoranylidine)acetate, CH_2Cl_2, room temperature (RT), 88% (two steps); b) 1. LiOH, THF/MeOH/H_2O; 2. TBS-Cl, Et_3N/DMAP; 3. $(COCl)_2$, DMF (cat.); c) 2,6-dibromoaniline, NaH, DMF/THF, 75 °C, 1.5 h, 44%; d) $[Pd(PPh_3)_4]$ or $Pd(OAc)_2$, 5–15%. DIBAL = diisobutylaluminum hydride, TBS = *tert*-butyl dimethylsilyl, DMAP = 4-dimethylaminopyridine.

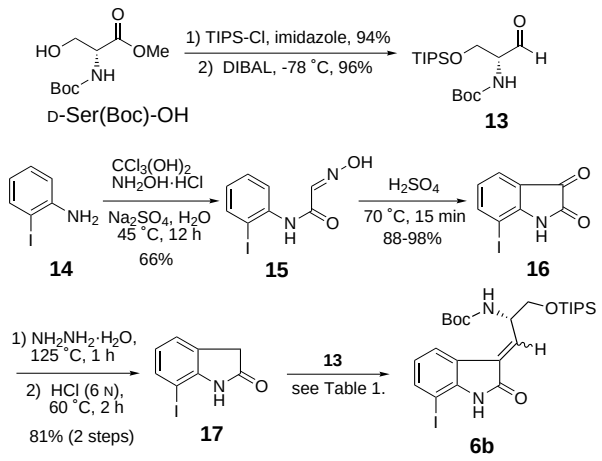
Thus, methyl ester **9** derived from D-serine[10] was reduced with DIBAL. This was followed by treatment with methyl (triphenylphosphoranylidine)acetate to provide **10** in 88% yield over two steps. Methyl ester **10** was then converted to the corresponding acid chloride **11** under neutral conditions in a three-step procedure as shown.[11] Acylation of 2,6-dibromoaniline with **11** was sluggish, and afforded **12** in only moderate yield. Unfortunately, attempted cyclization of dibromide **12** to 7-bromooxindole **6a** provided the desired compounds in unacceptably low yields (5–15%).

Accordingly, an alternative approach was investigated (Scheme 3). Protection of *N*-Boc-D-serine as shown, was followed by reduction of the methyl ester to provide aldehyde **13** in high yield. The required 7-iodoisatin **16** was obtained in good yield in two steps from 2-iodoaniline **14** via isonitrosoacetanilide **15**.[12] Upon heating compound **16** with hydrazine hydrate[13] for 1 h at 125 °C, and subsequent treatment with

[*] Prof. S. J. Danishefsky,[+] Dr. S. Lin
Laboratory for Bioorganic Chemistry
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, New York, NY 10021 (USA)
Fax: (+1) 212-772-8691
E-mail: s-danishefsky@ski.mskcc.org

[+] Department of Chemistry
Havemeyer Hall, Columbia University
New York, NY 10027 (USA)

[**] This work was supported by grants from the National Institutes of Health (grant CA28824). We thank Dr. George Sukenick and Sylvi Rusli of the MSKCC NMR Core Facility for NMR and mass spectral analyses (NIH Grant CA08748).



Scheme 3. Synthesis of 7-iodooxindole **6b**.

HCl (6 N) at 60 °C for 2 h, 7-iodooxindole **17** was obtained in excellent yield. Condensation of **17** and aldehyde **13** was then carried out under several conditions as summarized in Table 1. As seen, serviceable yields (74–76 %) with little or no racemization (up to 96 % *ee*) could be achieved in reaching **6b** (Table 1, entries 3 and 4).[14] The structure of the major component of **6b** was confirmed by NOE experiments (Figure 1) to be the *E* isomer.

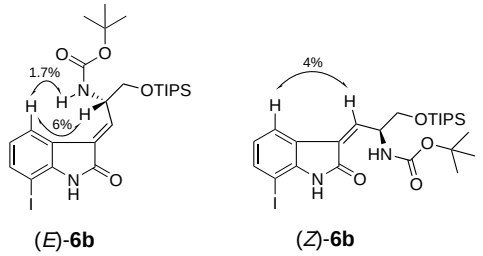
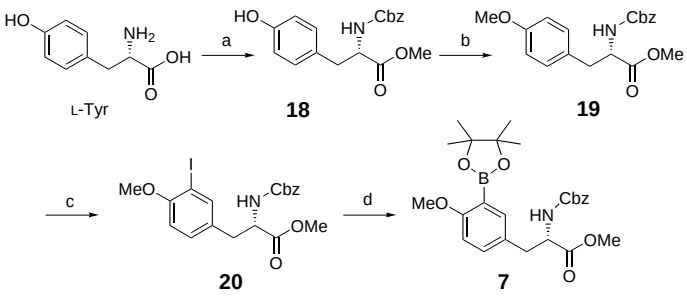


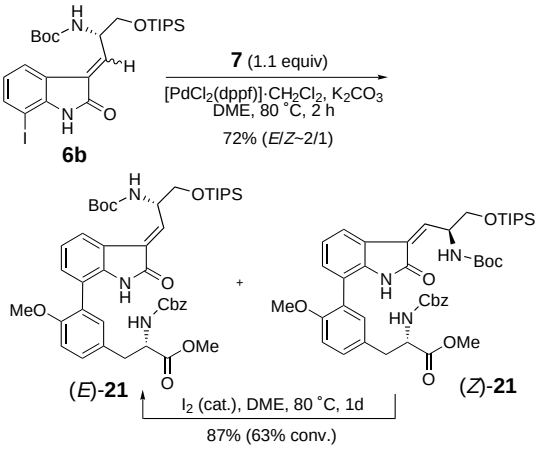
Figure 1. NOE experiments on compounds (*E*)-**6b** and (*Z*)-**6b** ($CDCl_3$, 400 MHz).

The aryl borate **7**, required for Suzuki cross-coupling, was synthesized starting from L-tyrosine (Scheme 4). Methyl ester formation with $MeOH/SOCl_2$ followed by protection of the amino group as a benzylcarbamate, afforded phenol **18** in 96 % yield over two steps. O-methylation of **18** with Me_2SO_4 in the presence of $LiOH \cdot H_2O$ in dry THF[15] provided **19** in 86 % yield. Selective iodination of **19** at the 3-position *ortho* to the methoxy group was achieved in high yield with $I_2/AgSO_4$[16] in methanol. Iodide **20** was then converted into aryl borate **7** in 95 % yield under Miyaura's conditions using bis(pinacolato)diboron, $[PdCl_2(dppf)] \cdot CH_2Cl_2$, and KOAc in DMSO for 13 h at 80 °C.[17]



Scheme 4. Synthesis of aryl borate **7**. a) 1. $MeOH/SOCl_2$; 2. Cbz-Cl, K_2CO_3, H_2O/acetone, 96 % (two steps); b) LiOH, Me_2SO_4, 86 %; c) I_2, Ag_2SO_4, MeOH, RT, 1.5 h; 93 %; d) bis(pinacolato)diboron, $[PdCl_2(dppf)] \cdot CH_2Cl_2$, KOAc, DMSO, 80 °C, 13 h, 95 %. dppf = bis(diphenylphosphanyl)ferrocene.

The appropriate components, 7-iodooxindole **6b** and aryl borate **7**, were indeed joined by a Suzuki coupling.[9, 17b] After an extensive survey of reaction conditions (catalyst loading, temperature, and reaction time, equivalents of borate and base) we identified a regimen that afforded coupling products **21** in 72 % yield with an *E*/*Z* ratio of 2/1. The two isomers were separable by silica gel chromatography, and the *E*/*Z* stereochemistry of each was determined by NOE experiments similar to those conducted on **6b**. No epimerization of the allylic amine position was observed in this reaction. However, *E*/*Z* isomerization apparently did occur during the Suzuki process. An *E*/*Z* ratio of about 2/1 was consistently obtained starting with (*E*)-**6b**, (*Z*)-**6b**, or a mixture of the two isomers. Conversion of (*Z*)-**21** to (*E*)-**21** can be affected by heating (*Z*)-**21** in DME at 80 °C for 1 day in the presence of a catalytic amount of iodine (Scheme 5).



Scheme 5. Biaryl linkage formation by Suzuki coupling. DME = 1,2-dimethoxyethane.

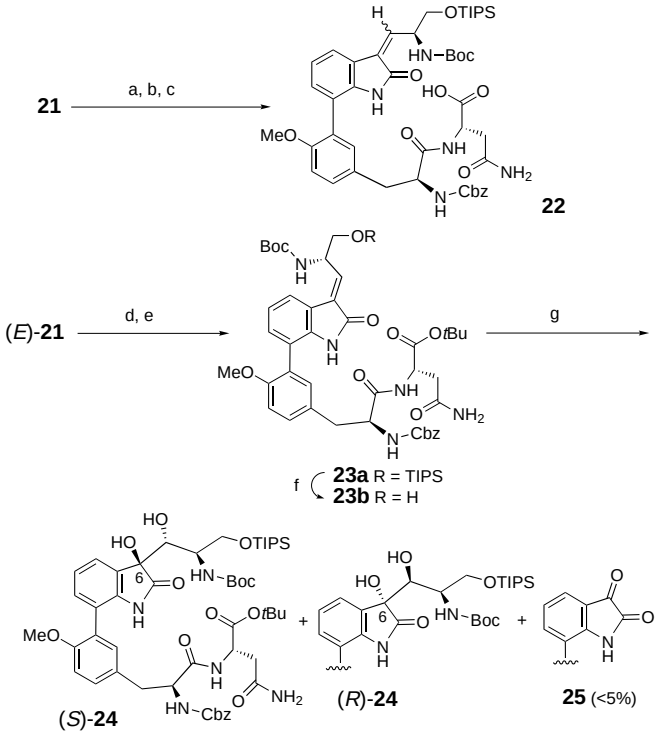
Table 1. Condensation of iodooxindole **17** with aldehyde **13** under different conditions.

Entry	Conditions	Yield [%][a]	*E*/*Z*[b]	*ee* [%][c]
1	piperidine (cat.), MeOH or EtOH, 65 °C, 2–3 h	44–50	2.0/1	0
2	piperidine (cat.), THF, RT, 17–44 h	55	1.7/1	~10
3	1) LDA (2.1 equiv), THF, −78 °C, 1 h; 2) TEA (2.5 equiv), MsCl (1.2 equiv), CH_2Cl_2, −60 to −30 °C, 2 h	76	1.3/1	92
4	1) LDA (2.0 equiv), THF, −78 °C, 1.5 h; 2) TEA (3 equiv), MsCl (1.5 equiv), CH_2Cl_2, −70 to −50 °C, 1.5 h	74	1.2/1	96

[a] Yield of isolated product. [b] Determined by ^1H NMR spectroscopy, two isomers are separable. [c] Determined by chiral HPLC.

  0044-8249/01/11310-2022 $ 17.50+.50/0 *Angew. Chem.* **2001**, *113*, Nr. 10

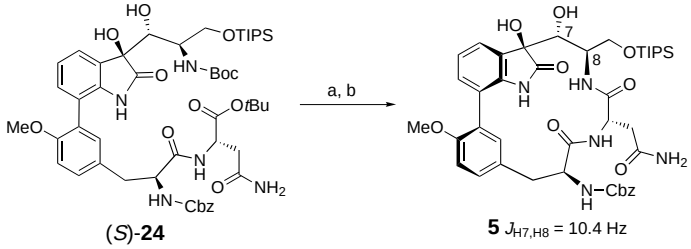
L-Asparagine was then appended to the seco framework. Thus, hydrolysis of the α-methyl ester of **21**, and conversion of the derived acid to its *N*-hydroxysuccinimade ester paved the way for amide formation with L-Asn hydrate (see compound **22**). Exposure of **22** to the action of HCl (4 N), followed by cyclization of the resulting amino acid with pentafluorophenyl diphenylphosphonate (FDPP) and DIEA in DMF, or with EDC/HOAT in various solvents (DMF, CH_2Cl_2, MeCN), however, did not provide the cyclized product. We reasoned that the rigidity of the *exo* double bond at the 3-position of oxindole ring probably tilts the amino group away from the asparagine moiety, thereby preventing cyclization. Accordingly, we decided to carry out dihydroxylation prior to cyclization. Saponification of the methyl ester (*E*)-**21** followed by coupling with L-asparagine *tert*-butyl ester (**8**) as before provided **23a** in 70% yield over two steps. Treatment of **23a** with HF/pyridine afforded free alcohol **23b** (Scheme 6).



Scheme 6. Synthesis of diols **24**. a) LiOH, THF/MeOH/H_2O; b) hydroxysuccinimade, DCC, THF, 55% (two steps); c) L-Asn·H_2O, Et_3N, THF/H_2O, RT, 4 h, 70%; d) LiOH, THF/H_2O, 0 °C, 1.5 h; e) H-Asn-O*t*Bu (**8**), EDC/HOAT, THF, RT, 2 h, 70% (2 steps); f) HF/Py, 84%; g) 1: OsO_4/NMO, $(DHQD)_2PHAL$, *t*BuOH/H_2O, RT, 1 h, 84% (*S*/*R* ~ 1/1.8); 2: OsO_4/NMO, $(DHQ)_2PHAL$, *t*BuOH/H_2O, RT, 4 h; TIPS-Cl, imidazole/DMAP, 5 h, 81% (*S*/*R* ~ 1/1.4). DCC = 1,3-dicyclohexylcarbodiimide, EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, HOAT = 1-hydroxy-7-azabenzotriazole, Asn = asparagine, NMO = 4-methylmorpholine *N*-oxide, $(DHQD)_2PHAL$ = 1,4-bis(9-*O*-dihydroquinidine)phthalazine, $(DHQ)_2PHAL$ = 1,4-bis(9-*O*-dihydroquinine)phthalazine.

Dihydroxylation of **23a** using OsO_4/NMO in the presence of $(DHQD)_2PHAL$[18] at room temperature afforded the diols **24** in 84% yield (*S*/*R* ~ 1/1.8),[19] along with a small amount of isatin **25** (< 5%). Dihydroxylation of **23b** in the presence of $(DHQ)_2PHAL$[18] followed by selective reprotection of the primary hydroxy group gave similar results (81%, *S*/*R* ~ 1/1.4, Scheme 6).

Treatment of (*S*)-**24** with trifluoroacetic acid (TFA) in CH_2Cl_2 resulted in concurrent removal of the Boc protecting group and hydrolysis of the *tert*-butyl ester. The crude amino acid was then submitted to macrolactamization using EDC/HOAT under highly dilute conditions (4 mM) in CH_2Cl_2/DMF (4/1). Cyclization progressed smoothly, providing the desired product **5**[20] in 55% yield over two steps (Scheme 7). The



Scheme 7. Macrolactamization of (*S*)-**24**. a) TFA/CH_2Cl_2 (4/1), RT, 2 h; b) EDC, HOAT, DIEA, CH_2Cl_2/DMF (4/1, 4 mM), RT, 20 h, 55% (two steps). DIEA = *N*,*N*-diisopropylethylamine.

large coupling constant (10.4 Hz) observed for H7 – H8 in **5**, which is similar to those observed in TMC-95A and B (**1** and **2**),[2] further confirmed the configurational assignments at C6 and C7. Interestingly, treatment of (*R*)-**24** under the same reaction conditions did not afford any cyclization product.

In summary, the fully functionalized macrocyclic core of proteasome inhibitors TMC-95A and B (**5**) has been assembled. There still remain significant issues to be overcome, such as selectivity enhancement en route to our total synthesis goal,[21] which are currently being studied in considerable detail.[22]

Received: March 14, 2001 [Z16776]

[1] Y. Koguchi, J. Kohno, M. Nishio, K. Takahashi, T. Okuda, T. Ohnuki, S. Komatsubara, *J. Antibiot.* **2000**, *53*, 105 – 109.

[2] J. Kohno, Y. Koguchi, M. Nishio, K. Nakao, M. Kuroda, R. Shimizu, T. Ohnuki, S. Komatsubara, *J. Org. Chem.* **2000**, *65*, 990 – 995.

[3] A. Ciechanover, A. L. Schwartz, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2727 – 2730.

[4] M. Orlowski, C. Michaud, *Biochemistry* **1989**, *28*, 9270 – 9278.

[5] K. Matsuzaki, H. Ikeda, T. Ogino, A. Matsumoto, H. B. Woodruff, H. Tanaka, S. Õmura, *J. Antibiot.* **1994**, *47*, 1173 – 1174.

[6] I. Kaneko, K. Kamoshida, S. Takahashi, *J. Antibiot.* **1989**, *42*, 236 – 241.

[7] N. Lindquist, W. Fenical, G. D. Van Duyne, J. Clardy, *J. Am. Chem. Soc.* **1991**, *113*, 2303 – 2304.

[8] a) N. Naruse, O. Tenmyo, S. Kobaru, M. Hatori, K. Tomita, Y. Hamagishi, T. Oki, J. Careofokumura, *J. Antibiot.* **1993**, *46*, 1804 – 1811; b) N. Naruse, M. Oka, M. Konishi, T. Oki, *J. Antibiot.* **1993**, *46*, 1812 – 1818.

[9] For a review of Suzuki coupling reactions, see: N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457 – 2483.

[10] A. McKillop, R. K. Taylor, R. J. Watson, N. Lewis, *Synthesis* **1994**, 31 – 33.

[11] A. Wissner, C. V. Grudzinskas, *J. Org. Chem.* **1978**, *43*, 3972 – 3974.

[12] a) C. S. Marvel, G. S. Hiers, *Org. Synth.* **1941**, *Coll. Vol. I*, 327 – 330; b) M. S. Newman, M. W. Logue, *J. Org. Chem.* **1971**, *36*, 1398 – 1401; c) V. Lisowski, M. Robba, S. Rault, *J. Org. Chem.* **2000**, *65*, 4193 – 4194.

[13] C. Crestini, R. Saladino, *Synth. Commun.* **1994**, *24*, 2835 – 2841.

[14] Related synthetic studies on TMC-95A – D had been reported by Ma and Wu who employed an oxindole addition/elimination – iodine equilibration – dihydroxylation approach to a portion of macrocyclization precursor: D. Ma, Q. Wu, *Tetrahedron Lett.* **2000**, *41*, 9089 – 9093.

[15] A. Basak, M. K. Nayak, A. K. Chakraboti, *Tetrahedron Lett.* **1998**, *39*, 4883 – 4886.

[16] W. W. Sy, *Tetrahedron Lett.* **1993**, *34*, 6223 – 6224.

[17] a) T. Ishiyama, M. Murata, N. Miyaura, *J. Org. Chem.* **1995**, *60*, 7508 – 7510; b) A. M. Elder, D. H. Rich, *Org. Lett.* **1999**, *1*, 1443 – 1446.

[18] K. B. Sharpless, W. Amberg, Y. L. Bennani, G. A. Crispino, J. Hartung, K.-S. Jeong, H.-L. Kwong, K. Morikawa, Z.-M. Wang, D. Xu, X.-L. Zhang, *J. Org. Chem.* **1992**, *57*, 2768 – 2771.

[19] The *R* configuration at C6 of (*R*)-**24** was assigned by converting (*R*)-**24** to its primary – secondary diol acetonide, whereupon the coupling constant between H7 and H8 is 0 Hz, indicating their *syn* relationship.

[20] The atropisomer shown for **5** follows from the C6 stereochemistry (see reference [2]).

[21] While the manuscript was being processed, it has been found that dihydroxylation of the *N*,*O*-acetonide derivative of **23b** under similar conditions to those shown in Scheme 6 followed by protecting group adjustment provided the desired (*S*)-**24** as the major isomer (*S*/*R* ~ 8/1). Details will be reported in due course. Similar results have also been observed for **6a** (see reference [14]).

[22] **Note added in proof** (April 12, 2001): An alternative route to the biaryl moiety of the TMC-95 natural products by a Pd-catalyzed Stille cross-coupling reaction of an aryl stannane tyrosine derivative and 7-iodoisatin appeared following the acceptance of this work: B. K. Albrecht, R. M. Williams, *Tetrahedron Lett.* **2001**, *42*, 2755 – 2757.

  0044-8249/01/11310-2024 $ 17.50+.50/0