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The Synthesis and Inhibitory Activity of Dethiotrypanothione and Analogues against Trypanothione Reductase

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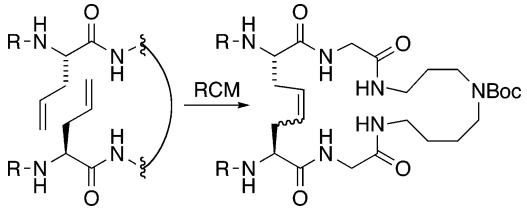
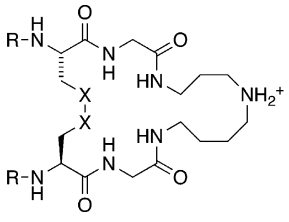


Trypanothione reductase (TR) catalyzes the NADPH-dependent reduction of trypanothione disulfide (**1**). TR plays a central role in the trypanosomatid parasite's defense against oxidative stress and has emerged as a promising target for antitrypanosomal drugs. We describe the synthesis and activity of dethiotrypanothione and analogues (**2**−**4**) as inhibitors of *Trypanosoma cruzi* TR. The syntheses of these macrocycles feature ring-closing olefin metathesis (RCM) reactions catalyzed by ruthenium catalyst **17**. Derivative **4** is our most potent inhibitor with a $K_i = 16\ \mu M$.

The trypanosomatid parasites are the causative agents of several tropical diseases in both humans and domestic animals. Examples include Chagas' disease (*Trypanosoma cruzi*), African sleeping sickness (*T. brucei gambiense* and *T.b. rhodensiense*, West African and East African trypanosomiasis, respectively), and nagana cattle disease (*T.b. brucei*, *T. conglense*).[1] These parasites have a unique thiol metabolism centered on the NADPH-dependent flavoenzyme trypanothione reductase (TR), which catalyzes the reduction of the antioxidant trypanothione[2] (**1**, N^1,N^8-bis(glutathionyl)spermidine) from its disulfide to its dithiol form.[3] The parasites rely on the trypanothione/TR system to maintain an intracellular reducing environment and to combat oxidative stress,[4] and several genetic studies have demonstrated the parasites' dependence on TR for growth and virulence.[5]

In the trypanosomatids, the trypanothione/TR couple replaces the closely related glutathione/glutathione reductase (GR) system found in host organisms.[3] TR and GR display a high degree of sequence[3] and structural homology,[6] the catalytic mechanisms of both enzymes are essentially identical,[7] and yet the enzymes display almost complete specificity for their respective substrates.[8] This mutually exclusive substrate specificity between the parasite and host enzymes, and the parasite's sensitivity to a loss of TR function, combine to make TR a promising drug target. Accordingly, a large number of TR inhibitors have been reported.[4,9]



1: R = γ-glutamyl, X = S (trypanothione disulfide)
2: R = γ-glutamyl, X = CH_2 (dethiotrypanothione)
3: R = Cbz, X = CH_2
4: R = Fmoc, X = CH_2

Here we report the synthesis and inhibitory activity of **2**−**4**, structural analogues of the natural substrate. Compound **2**, which we have named dethiotrypanothione, contains all of the structural elements of trypanothione, except that the substrate's redox-active disulfide moiety has been replaced by a pair of methylene

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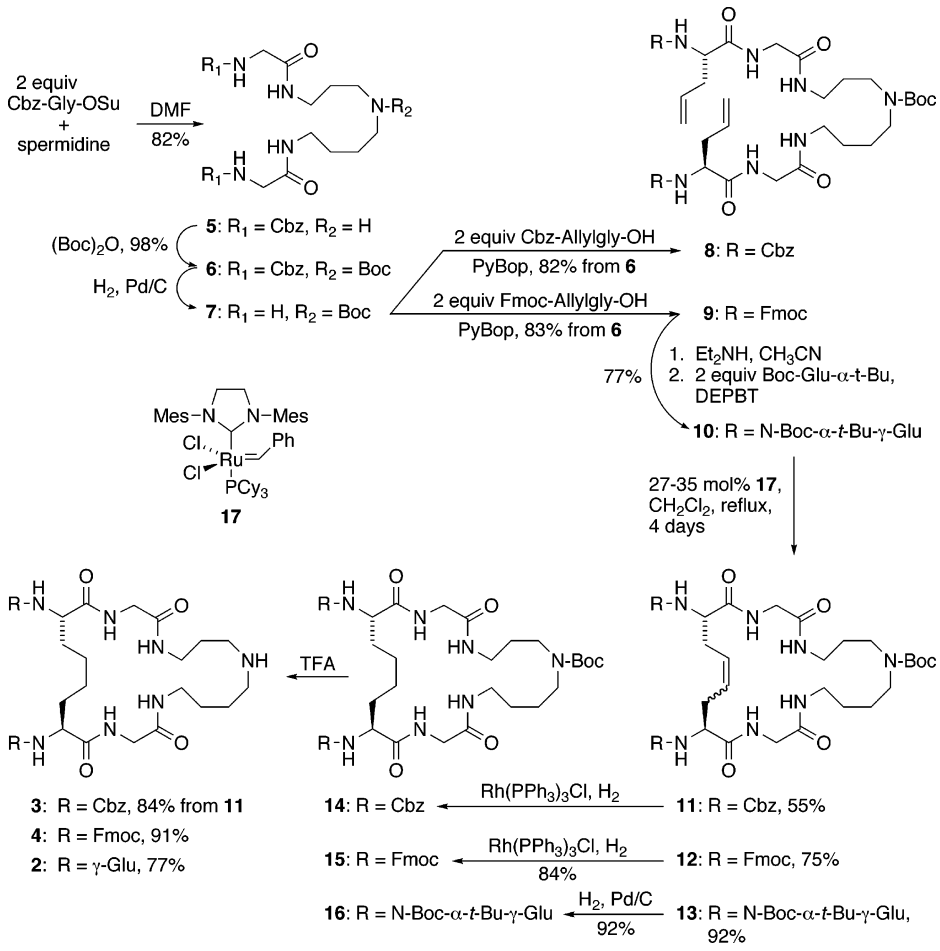
SCHEME 1. Synthesis of Trypanothione Analogues



groups. Analogues **3** and **4** also lack the disulfide, and in addition, trypanothione's γ-glutamyl groups have been replaced by hydrophobic aromatic moieties in these compounds. We prepared these analogues to explore the curious tolerance the enzyme displays for substrate analogues incorporating Cbz groups and other hydrophobic moieties in place of the zwitterionic glutamate residues.[10]

Our syntheses of dethiotrypanothione and analogues feature ring-closing olefin metathesis (RCM) reactions catalyzed by second-generation Grubb's catalyst **17**,[11] as shown in Scheme 1. RCM has emerged as a powerful tool in the formation of macrocyclic compounds due to the exceptional chemoselectivity of the recently developed catalysts (such as **17**) and their tolerance of diverse functionality.[12] RCM seemed particularly well-suited to the synthesis of our 24-membered ring macrocycles **2**−**4** since we envisioned that the required RCM substrates could be efficiently constructed from spermidine and commercially available amino acid derivatives, in a bidirectional fashion from common intermediate **7**.

The known diamine intermediate **7** is derived from its Cbz-protected derivative **6**, which was first reported by Henderson and co-workers in their synthesis of trypanothione.[13] Their four-step method afforded **6** in about 50% overall yield and proceeded through a hexahydropyrimidine derivative of spermidine developed by Ganem et al.,[14] which elegantly distinguishes the three amino groups of spermidine. We found that **6** is more efficiently prepared by direct treatment of spermidine with 2 equiv of Cbz-glycine *N*-hydroxysuccinimide ester. This reaction proceeded with selective acylation of the primary

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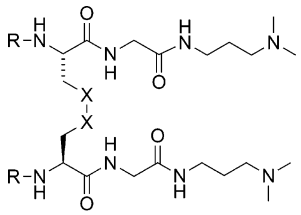
amines without detectable modification of spermidine's secondary nitrogen.[15] Subsequent blocking of the secondary amine with the Boc group provided **6** in just two steps from spermidine, with an 80% overall yield.

Hydrogenolysis of the Cbz groups of **6** followed by PyBop-mediated[16] coupling of the resulting diamine **7** with 2 equiv of either Cbz-allylglycine or Fmoc-allylglycine provided **8** or **9**, respectively. Intermediates **8** and **9** were the substrates for the RCM reactions in the syntheses of inhibitors **3** and **4**, respectively. In the case of the synthesis of **2**, **9** was elaborated to the bis-γ-glutamyl RCM substrate **10**, using DEPBT[17] as the peptide coupling reagent. The RCM reactions of **8**−**10** were carried out under high dilution in refluxing CH_2Cl_2, in the presence of 27−35 mol % of catalyst **17**,[11] with yields ranging from 55 to 92%. Olefins **11**−**13** (*E*/*Z* mixtures) were reduced to their saturated derivatives **14**−**16**, and finally, the *tert*-butyl-based protecting groups of **14**, **15**, and **16** were removed under acidic conditions to afford inhibitors **3**, **4**, and **2**, respectively.

Trypanothione analogues **2**−**4** were evaluated as reversible inhibitors of recombinant *T. cruzi* TR.[18] TR activity was assayed using disulfide substrate **18**, developed by Douglas and co-workers, as an effective alternative to the expensive trypanothione.[19] The reaction was monitored spectrophotometrically by following the oxidation of NADPH at 340 nm.[20] The K_i values for each inhibitor are given in Table 1. Dethiotrypanothione proved to be a very poor inhibitor of the enzyme. On the other hand, replacing the charged γ-glutamyl moieties of **2** with Cbz groups (**3**) resulted in nearly 6-fold stronger binding, while **4**, with the larger Fmoc groups in place of the γ-glutamyl moieties, was our most potent inhibitor. A thorough analysis of the inhibitory activity of **4** was precluded by its low solubility under the assay conditions, restricting the concentration range over which we could evaluate this compound. Thus, our determination of a $K_i = 16\ \mu$M for **4** was based on assays using just two inhibitor concentrations (6.4 and 11.1 μM), each examined over a range of four substrate concentrations.[21] Since we were not able to assay **4** at concentrations above its determined K_i value, the precise value of 16 μM should be read with some caution. Nevertheless, it is clear that the Fmoc derivative binds TR with an affinity of about an order of magnitude greater than that of Cbz derivative **3**.



18: R = Cbz, X = S
19: R = Cbz, X = CH_2
20: R = γ-glutamyl, X = S

TABLE 1. Inhibition of *T. cruzi* TR[a]

entry	inhibitor	K_i (μM)
1	**2**	826 ± 76
2	**3**	145 ± 24
3	**4**	16 ± 2

[a] Our determination of K_m for assay substrate **18** was 6.7 μM.

We also tested our inhibitors for activity against yeast GR. For compounds **3** and **4**, no inhibition of GR was observed up to concentrations of 1.1 mM of **3** and 16 μM of **4** (the solubility limit for this compound). Dethiotrypanothione (**2**) at 5 mM showed no detectable inhibition of GR; however, at 10 mM **2**, we did observe about 20% inhibition compared to control assays, indicating that **2** does interact with GR at very high concentrations. Our lack of sufficient quantities of **2** precluded a more complete analysis of dethiotrypanothione's activity against GR. Nevertheless, all three inhibitors displayed decided selectivity for the parasite enzyme.

Given the structural similarity between dethiotrypanothione **2** and trypanothione disulfide ($K_m = 50\ \mu$M, $k_{cat} = 192\ s^{-1}$),[18] we did not expect **2** to be such a poor TR ligand. Of course, **2**−**4** do lack the substrate's disulfide group and the potential electrostatic binding contacts that this moiety may afford. Furthermore, the geometry of the disulfide moiety, which typically exhibits about a 90° dihedral angle about the S−S bond, would be expected to be quite different than the geometry of the methylene bridge in our inhibitors. We have previously reported the TR inhibitory activity of **19**, an inhibitor that also lacks the disulfide moiety.[10c] Inhibitor **19** ($K_i = 30\ \mu$M) was modeled after TR substrate **18** ($K_m = 6.7\ \mu$M). Interestingly, like **2** and trypanothione disulfide, **19** and **18** also differ only in the substitution of the disulfide group by a pair of methylenes, yet unlike **2** and trypanothione disulfide, the affinities of **19** and **18** for TR appear to be comparable.

We were not surprised by the affinity TR displayed for analogues **3** and **4** since it is well-established that TR tolerates hydrophobic groups in the place of the γ-glutamyl residues in alternate substrates such as **18**.[10] Douglas and co-workers explored the enzyme's tolerance for γ-glutamyl-modified substrates in their study comparing the activities of derivatives based on γ-glutamylcysteinylglycyl-3-dimethylaminopropylamide (**20**).[10a] While the Cbz-modified derivative **18** retains only 58% of the catalytic efficiency (k_{cat}/K_m) observed for **20**, **18** does have a significantly lower K_m than **20** (52 μM for **18** versus 225 μM for **20**),[22] suggesting stronger binding of the Cbz groups compared to the γ-glutamyl residues. On the basis of crystallographic data, Douglas' group proposed that a Cbz moiety binds

(15) We previously reported selective acylation of a primary over a secondary amine with succinimide esters; see ref 10c. Selective trifluoroacylation of the primary amino groups of spermidine and related polyamines has also been reported; see: O'Sullivan, M. C.; Dalrymple, D. M. *Tetrahedron Lett.* **1995**, *36*, 3451−3452.

(16) PyBop = benzotriazole-1-yloxytrispyrrolidinophosphonium hexafluorophosphate. Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205−208.

(17) DEPBT = 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one. Li, H.; Jiang, X.; Ye, Y.-h.; Fan, C.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91−93.

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(21) For **2**, four different inhibitor concentrations were used, varied from 0.70 to 10.3 mM. For **3**, three different inhibitor concentrations were examined, ranging from 228 to 912 μM. For all inhibitors, each inhibitor concentration was examined over a range of four substrate concentrations, with all assays performed in at least duplicate. See the Supporting Information for full experimental details.

(22) Douglas and co-workers used the enzyme from *C. fasciculata* in their study, which accounts for the difference in the K_m value they report for **18** (52 μM), compared to our determination of this parameter using the *T. cruzi* enzyme (6.7 μM).

in a hydrophobic pocket (the "Z-site") on the enzyme that is not utilized in the binding of the natural substrate.[9c,23] The more potent inhibition observed for our Fmoc derivative **4** suggests that this putative Z-site may favorably accommodate aromatic groups that are significantly larger than the Cbz moiety. On the other hand, in addition to the Z-site, the enzyme also possesses a hydrophobic patch comprising the side chains of Leu18, Trp22, Tyr111, and Met114, where trypanothione's spermidine moiety binds.[6] The aromatic acridine ring of a quinacrine-based inhibitor was recently shown to bind in this hydrophobic site,[24] and this pocket is also the putative binding site of a number of related hydrophobic tricyclic TR inhibitors.[25] It seems unlikely, but it is possible that **3** and **4** are turned around in the active site, binding in an orientation that places their aromatic moieties in this hydrophobic site that normally binds the substrate's spermidine moiety.

In conclusion, we have developed syntheses of dethiotrypanothione and related trypanothione analogues featuring RCM macrocyclizations. Dethiotrypanothione proved to be a very poor inhibitor of TR despite its close structural similarity to the natural substrate. However, the more hydrophobic analogues **3** and **4** are more effective inhibitors, underscoring the enzyme's general affinity for hydrophobic ligands.

Experimental Section

Dethiotrypanothione (2): ^{1}H NMR (400 MHz, CD_3OD) δ 4.26 (dd, 1H, J = 6.8, 6.8 Hz), 4.25 (dd, 1H, J = 5.9, 8.5 Hz), 4.04 (dd, 1H, J = 6.5, 6.5 Hz), 4.03 (dd, 1H, J = 6.3, 6.3 Hz), 3.88 (d, 1H, J = 16.1 Hz), 3.86 (d, 1H, J = 16.1 Hz), 3.77 (d, 1H, J = 16.1 Hz), 3.73 (d, 1H, J = 16.1 Hz), 3.40−3.33 (m, 2H), 3.32−3.24 (m, 2H), 3.03 (t, 2H, J = 6.8 Hz), 3.00 (t, 2H, J = 7.2 Hz), 2.60−2.53 (m, 4H), 2.29−2.10 (m, 4H), 1.93−1.84 (m, 2H), 1.84−1.66 (m, 6H), 1.65−1.57 (m, 2H), 1.51−1.35 (m, 2H) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 175.2, 175.0, 174.6, 174.5, 172.8, 171.9, 171.6, 55.0, 53.5, 45.7, 44.0, 43.8, 39.0, 36.5, 32.1, 27.3, 27.1, 26.9, 26.1, 25.7, 23.9 ppm; HRMS (ESI) m/z calcd for $C_{29}H_{52}N_9O_{10}$ [M + H]$^+$ 686.3832, found 686.3831.

Inhibitor 3: ^{1}H NMR (400 MHz, 15% $CH_3OD/CDCl_3$ with a trace of TFA-*d* added) δ 7.39−7.28 (m, 10H), 5.15−5.05 (m, 2H), 4.17−4.09 (m, 2H), 3.90−3.72 (m, 4H), 3.52−3.11 (m, 4H), 3.00−2.84 (m, 4H), 1.94−1.83 (m, 2H), 1.83−1.62 (m, 6H), 1.62−1.51 (m, 2H), 1.48−1.31 (m, 4H) ppm; ^{13}C NMR (100 MHz, 15% $CD_3OD/CDCl_3$ with a trace of TFA added) δ 174.7(C), 174.6(C), 172.0(C), 170.9(C), 157.62(C), 157.56(C), 136.9(C), 129.0(CH), 128.6(CH), 128.2(CH), 67.4(CH_2), 55.4(CH), 55.3(CH), 47.7(CH_2), 45.0(CH_2), 43.5(CH_2), 43.3(CH_2), 38.3(CH_2), 35.8(CH_2), 31.7(CH_2), 30.1(CH_2), 26.5(CH_2), 26.2(CH_2), 25.0(CH_2), 24.6(CH_2), 23.1(CH_2) ppm; HRMS (ESI) m/z calcd for $C_{35}H_{50}N_7O_8$ [M + H]$^+$ 696.3721, found 696.3738; m/z calcd for $C_{35}H_{49}N_7NaO_8$ [M + Na]$^+$ 718.3541, found 718.3557.

Inhibitor 4: ^{1}H NMR (400 MHz, 10% $CD_3OD/CDCl_3$ with ca. 3% TFA) δ 7.76 (d, 4H, J = 7.3 Hz), 7.62 (d, 4H, J = 6.6 Hz), 7.40 (t, 4H, J = 7.05 Hz), 7.31 (t, 4H, J = 6.8 Hz), 4.52−4.33 (m, 4H), 4.25−4.17 (m, 2H), 4.15−4.08 (m, 2H), 3.90−3.77 (m, 4H), 3.47−3.13 (m, 4H), 3.01−2.83 (m, 4H), 1.93−1.49 (m, 8H), 1.45−1.16 (m, 6H) ppm; ^{13}C NMR (100 MHz, 10% $CD_3OD/CDCl_3$ with ca. 1−2% TFA) δ 173.9, 173.8, 171.3, 170.2, 161.5, 156.7, 156.7, 143.4, 143.4, 141.0, 127.4, 126.7, 124.61, 124.57. 119.6, 77.2, 66.6, 54.5, 46.9, 46.8, 44.1, 42.7, 42.5, 37.5, 35.0, 30.9, 29.3, 25.6, 25.5, 24.1, 23.8, 22.2 ppm; HRMS (ESI) m/z calcd for $C_{49}H_{58}N_7O_8$ [M + H]$^+$ 872.4341, found 872.4317.

***N*^{1},*N*^{8}-Bis(benzyloxycarbonylglycyl)spermidine (5):** mp 148−149 °C (lit.[13] 149−150 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (t, 1H, J = 5.4 Hz), 7.86 (t, 1H, J = 5.7 Hz), 7.45−7.30 (m, 11.6H), 7.09−7.02 (m, 0.4H CONH, minor rotamer), 5.03 (s, 4H), 3.57 (d, 4H, J = 6.0 Hz), 3.09 (dt, 2H, J = 6.4, 6.4 Hz), 3.05 (dt, 2H, J = 6.2, 6.2 Hz), 2.48−2.43 (m, 4H), 1.52 (quint, 2H, J = 6.7 Hz), 1.44−1.33 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.8, 168.7, 156.4, 137.0, 136.9, 128.2, 127.7, 127.6, 65.4, 48.8, 46.7, 43.5, 38.4, 36.9, 29.2, 26.9, 26.7 ppm; LRMS (ESI) m/z 528.4 [M + H]$^+$, 550.3 [M + Na]$^+$.

***N*^{1},*N*^{8}-Bis(benzyloxycarbonylglycyl)-*N*^{4}-*tert*-butoxycarbonylspermidine (6):** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.25 (m, 10.7H), 6.93−6.70 (m, 1.3H), 6.14−5.90 (m, 2H), 5.09 (s, 2H), 5.08 (s, 2H), 3.84−3.81 (m, 4H), 3.26−3.05 (m, 8H), 1.71−1.56 (m, 2H), 1.54−1.39 (m, 4H), 1.42 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4(C), 156.8(C), 156.1(C), 136.3(C), 136.2(C), 128.51(CH), 128.47(CH), 128.2(CH), 128.1(CH), 128.0(CH), 79.7(C), 67.0(CH_2), 66.8(CH_2), 46.8(CH_2), 45.1(CH_2), 44.5(CH_2), 43.9(CH_2), 38.8(CH_2), 36.2(CH_2), 29.3(CH_3, *tert*-butyl, minor rotamer), 28.4(CH_3), 27.8(CH_2), 26.7(CH_2), 25.8(CH_2) ppm; LRMS (ESI) m/z 650.3 (M + Na)$^+$.

Diene 8: ^{1}H NMR (400 MHz, $CDCl_3$,) δ 7.73−6.87 (m, 14H), 6.06−5.62 (m, 4H), 5.19−4.98 (m, 8H), 4.40−4.16 (m, 2H), 4.10−3.66 (m, 4H), 3.36−3.00 (m, 8H), 2.69−2.34 (m, 4H), 1.77−1.60 (m, 2H), 1.59−1.33 (m, 13H, within this multiplet lies the *tert*-butyl group C*H*$_3$ singlet at 1.42 ppm) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1(C), 169.0(C), 156.5(C), 156.1(C), 136.1(C), 136.0(C), 133.0(CH), 132.7(CH), 128.6(CH), 128.5(CH), 128.3(CH), 128.2(CH), 128.0(CH), 119.2(CH_2), 119.0(CH_2), 79.7(C), 67.13(CH_2), 67.10(CH_2), 54.7(CH), 47.0(CH_2), 45.3(CH_2), 44.1(CH_2), 43.2(CH_2), 38.7(CH_2), 36.6(CH_2), 28.4(CH_3), 27.8(CH_2), 26.6(CH_2), 25.9(CH_2); HRMS (ESI) m/z calcd for $C_{42}H_{59}N_7NaO_{10}$ [M + Na]$^+$ 844.4227, found 844.4217.

Diene 9: ^{1}H NMR (400 MHz, acetone-d_6) δ 7.91−7.83 (m, 2H CON*H*), 7.86 (d, 4H, J = 7.6 Hz), 7.69 (dd, 4H, J = 3.8, 7.5 Hz), 7.41 (t, 4H, J = 7.5 Hz), 7.41−7.24 (m, 2H, N*H*), 7.32 (t, 4H, J = 7.6 Hz), 6.95 (d, 2H, J = 7.0 Hz), 5.85 (dddd, 2H, J = 7.0, 7.0, 10.2, 17.1 Hz), 5.15 (dddd, 2H, J = 1.4, 1.4, 3.2, 17.1 Hz), 5.06 (dddd, 2H, J = 1.0, 2.1, 3.2, 10.2 Hz), 4.43−4.35 (m, 2H), 4.35−4.28 (m, 2H), 4.26−4.17 (m, 4H), 3.92 (dd, 2H, J = 6.0, 16.7 Hz), 3.82−3.73 (m, 2H), 3.24−3.07 (m, 8H), 2.69−2.58 (m, 2H), 2.53−2.43 (m, 2H), 1.74−1.59 (m, 2H), 1.52−1.40 (m, 4H), 1.39 (s, 9H) ppm; ^{13}C NMR (100 MHz, acetone-d_6) δ 172.7(C), 169.7(C), 157.4(C), 156.2(C), 144.9(C), 144.8(C), 142.0(C), 135.0(CH), 134.9(CH), 128.5(CH), 127.9(CH), 126.1(CH), 120.7(CH), 118.3(CH_2), 79.3(C), 67.4(CH_2), 56.0(CH), 55.5(CH_2), 47.9(CH), 47.4(CH_2), 45.0(CH_2), 43.6(CH_2), 43.5(CH_2), 39.3(CH_2), 36.9(CH_2), 32.0(CH_3), 28.6(CH_3), 26.54(CH_2) ppm; HRMS (ESI) m/z calcd for $C_{56}H_{67}N_7NaO_{10}$ [M + Na]$^+$ 1020.4847, found 1020.4851.

Diene 10: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98−7.61 (m, 2H), 7.49−7.06 (m, 4H), 6.12−5.86 (m, 0.4H, N*H* minor rotamer), 5.77 (dddd, 2H, J = 7.1, 7.1, 10.0, 17.1 Hz), 5.57−5.46 (m, 1.6H, N*H* major rotamer), 5.15 (d, 2H, J = 17.0 Hz), 5.11 (d, 2H, J = 10.1 Hz), 4.59−4.36 (m, 2H), 4.18−4.07 (m, 2H), 4.04−3.81 (m, 4H), 3.35−3.09 (m, 8H), 2.67−2.56 (m, 2H), 2.56−2.43 (m, 2H), 2.41−2.29 (m, 4H), 2.19−2.05 (m, 2H), 1.96−1.78 (m, 2H), 1.61−1.47 (m, 2H), 1.59−1.48 (m, 4H), [1.46 (s), 1.43 (s), 1.43 (s), 45H total] ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9(C), 172.0(C), 171.6(C), 169.2(C), 155.9(C), 133.4(CH), 133.3(CH), 118.7(CH_2), 118.6(CH_2), 82.1(C), 79.8(C), 79.5(C), 69.6(C), 54.2(CH_2), 53.6(CH), 53.4(CH), 53.2(CH), 47.0(CH_2), 43.2(CH_2), 38.8(CH_2), 36.2(CH_2), 32.1(CH_2), 31.8(CH_3), 30.9(CH_3), 29.3(CH_3), 28.7(CH_2), 28.5(CH_3), 28.4(CH_3), 28.0(CH_3), 26.5(CH_2), 25.8(CH_2) ppm; HRMS (ESI) m/z calcd for $C_{54}H_{93}N_9NaO_{16}$ [M + Na]$^+$ 1146.6633, found 1146.6594.

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General RCM Synthesis of Macrocycles 11, 12, and 13. Under an argon atmosphere, a solution of ruthenium catalyst **17** (27−35 mol %) was added to a dilute solution (2−3 mM) of the diene substrate (**8**, **9**, or **10)** in CH_2Cl_2, which had been degassed by bubbling argon through the mixture for 20−30 min. The resulting solution was gently refluxed for 4 days. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (for **11**, two columns were required to remove all traces of the catalyst, 5−10% CH_3OH/CH_2Cl_2 and then 60−80% acetone/hexane, for **12**, 40−100% acetone/toluene, and for **13**, 5−10% methanol/CH_2Cl_2).

Macrocycle 11: (55% yield); ^{1}H NMR (400 MHz, 5% $CD_3OD/CDCl_3$) δ 7.41−7.23 (m, 10H), 6.61−5.33 (m, 2H), 5.18−4.97 (m, 4H), 4.36−4.10 (m, 2H), 3.97−3.65 (m, 4H), 3.36−2.92 (m, 8H), 2.58−2.30 (m, 4H), 1.93−1.80 (m, 2H), 1.78−1.56 (m, 2H), 1.54−1.24 (m, 11H) ppm; ^{13}C NMR (100 MHz, 5% $CD_3OD/CDCl_3$) δ 172.7, 169.7, 156.7, 136.2, 128.6, 128.37, 128.35, 128.30, 128.2, 128.1, 128.0, 127.3, 80.0, 67.2, 55.1, 54.2, 46.8, 42.8, 38.7, 35.5, 35.3, 34.9, 31.8, 29.7, 29.2, 28.4, 27.0, 26.8, 26.31, 26.26, 26.22, 26.12, 25.4 ppm; HRMS (ESI) *m*/*z* calcd for $C_{40}H_{55}N_7NaO_{10}$ [M + Na]$^+$ 816.3908, found 816.3927.

Macrocycle 12: (75% yield); ^{1}H NMR (400 MHz, 1:1 $CD_3OD/CDCl_3$) δ 7.78 (d, 4H, J = 7.5 Hz), 7.67−7.57 (m, 4H), 7.40 (t, 4H, J = 7.4 Hz), 7.31 (t, 4H, J = 7.4 Hz), 5.58−5.30 (m, 2H), 4.53−4.33 (m, 4H), 4.26−4.07 (m, 4H), 3.94−3.73 (m, 4H), 3.32−3.30 (m, 8H), 2.55−2.32 (m, 4H), 1.78−1.63 (m, 2H), 1.56−1.37 (m, 13H); LRMS (ESI) *m*/*z* [M + Na]$^+$ 992.4; HRMS (FAB) *m*/*z* calcd for $C_{54}H_{63}N_7NaO_{10}$ [M + Na]$^+$ 992.4534, found 992.4522.

Macrocycle 13: (92% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12−7.64 (m, 2H), 7.56−7.08 (m, 4H), 5.70−5.35 (m, 4H), 4.77−4.25 (m, 2H), 4.20−3.59 (m, 6H), 3.44−3.00 (m, 8H), 2.81−2.29 (m, 8H), 2.26−2.00 (m, 2H), 1.97−1.64 (m, 4H), 1.64−1.29 (m, 49H) ppm; ^{1}H NMR (400 MHz, CD_3OD) δ 5.65−5.47 (m, 2H), 4.39−4.15 (m, 2H), 4.05−3.94 (m, 2H), 3.93−3.71 (m, 4H), 3.32−3.12 (m, 8H), 2.54−2.32 (m, 8H), 2.20−2.02 (m, 2H), 1.91−1.69 (m, 4H), 1.64−1.41 (m, 4H), [1.46 (s), 1.45 (s), 1.44 (s), 45H total] ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 175.0, 174.9, 174.1, 174.0, 173.3, 171.5, 171.4, 158.1, 157.3, 130.0, 129.8, 82.7, 81.4, 80.8, 80.5, 56.5, 56.1, 55.3, 55.2, 48.2, 46.4, 45.8, 43.6, 39.8, 38.0, 35.8, 35.5, 32.7, 29.6, 28.8, 28.3, 27.4 ppm; LRMS (ESI) *m*/*z* 1118.6 ([M + Na]$^+$, 100%), 1134.6 ([M + K]$^+$, 25%); HRMS (FAB) *m*/*z* calcd for $C_{52}H_{89}N_9Na_2O_{16}$ [M + Na]$^{2+}$ 570.8106, found 570.8123 (adding sample and reference together caused singly charged peaks to disappear, therefore, doubly charged (2 Na$^+$) peaks were used to measure exact mass).

Compound 15: ^{1}H NMR (400 MHz, 10% $CD_3OD/CDCl_3$) δ 7.76 (d, 4H, J = 7.5 Hz), 7.59 (d, 4H, J = 7.3 Hz), 7.40 (t, 4H, J = 7.5 Hz), 7.31 (t, 4H, J = 7.5 Hz), 4.54−4.30 (m, 4H), 4.20 (t, 2H, J = 6.5 Hz), 4.20−4.07 (m, 2H), 4.01−3.84 (m, 2H), 3.81−3.70 (m, 2H), 3.41−2.91 (m, 8H), 1.88−1.57 (m, 6H), 1.53−1.25 (m, 17H) ppm; ^{13}C NMR (100 MHz, 10% $CD_3OD/CDCl_3$) δ 173.0-(C), 169.6(C), 156.5(C), 156.4(C), 156.1(C), 155.6(C), 143.5(C), 141.09(C), 141.06(C), 127.6(CH), 126.9(CH), 124.7(CH), 119.8-(CH), 79.7(C), 77.2(CH), 66.7(CH_2), 66.5(CH_2), 54.4(CH_2), 54.1-(CH_2), 47.0(CH), 46.9(CH), 46.4(CH_2), 45.1(CH_2), 43.4(CH_2), 42.8(CH_2), 42.7(CH_2), 38.6(CH_2), 38.5(CH_2), 36.9(CH_2), 35.9(CH_2), 31.1(CH_2), 30.9(CH_2), 29.4(CH_2), 28.1(CH_3), 27.1(CH_2), 25.9(CH_2), 25.0(CH_2), 24.0(CH_2) ppm; HRMS (ESI) *m*/*z* calcd for $C_{54}H_{65}N_7-NaO_{10}$ [M + Na]$^+$ 994.4691, found 994.4680.

Compound 16: ^{1}H NMR (400 MHz, ca. 1:1 $CDCl_3/CD_3OD$) δ 4.42−4.09 (m, 2H), 4.05−3.66 (m, 6H), 3.35−3.00 (m, 8H), 2.44−2.22 (m, 4H), 2.18−1.97 (m, 2H), 1.95−1.62 (m, 8H), 1.90−1.65 (m, 8H), 1.62−1.15 (m, 45H) ppm; ^{13}C NMR (100 MHz, ca. 1:1 $CD_3OD/CDCl_3$) δ 174.3, 174.2, 171.0, 157.3, 157.0, 156.9, 156.8, 82.6, 81.1, 80.4, 80.3, 78.9, 56.0, 54.7, 54.3, 47.8, 46.2, 45.1, 43.6, 39.6, 39.5, 37.8, 37.3, 36.1, 35.5, 32.4, 31.9, 31.5, 29.3, 28.9, 28.8, 28.4, 27.8, 27.6, 27.4, 26.9, 26.9, 26.8, 26.3, 25.9, 25.2, 24.6 ppm; LRMS (ESI) *m*/*z* 1120.6 [M + Na]$^+$; HRMS (ESI) *m*/*z* calcd for $C_{52}H_{91}N_9NaO_{16}$ [M + Na] $^+$ 1120.6476, found 1120.6475.

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Supporting Information Available: Complete experimental details, analytical data, and NMR spectra for all compounds, and experimental details for the TR and GR assays of compounds **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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