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Tabun scavengers based on hydroxamic acid containing cyclodextrins

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1. Structures

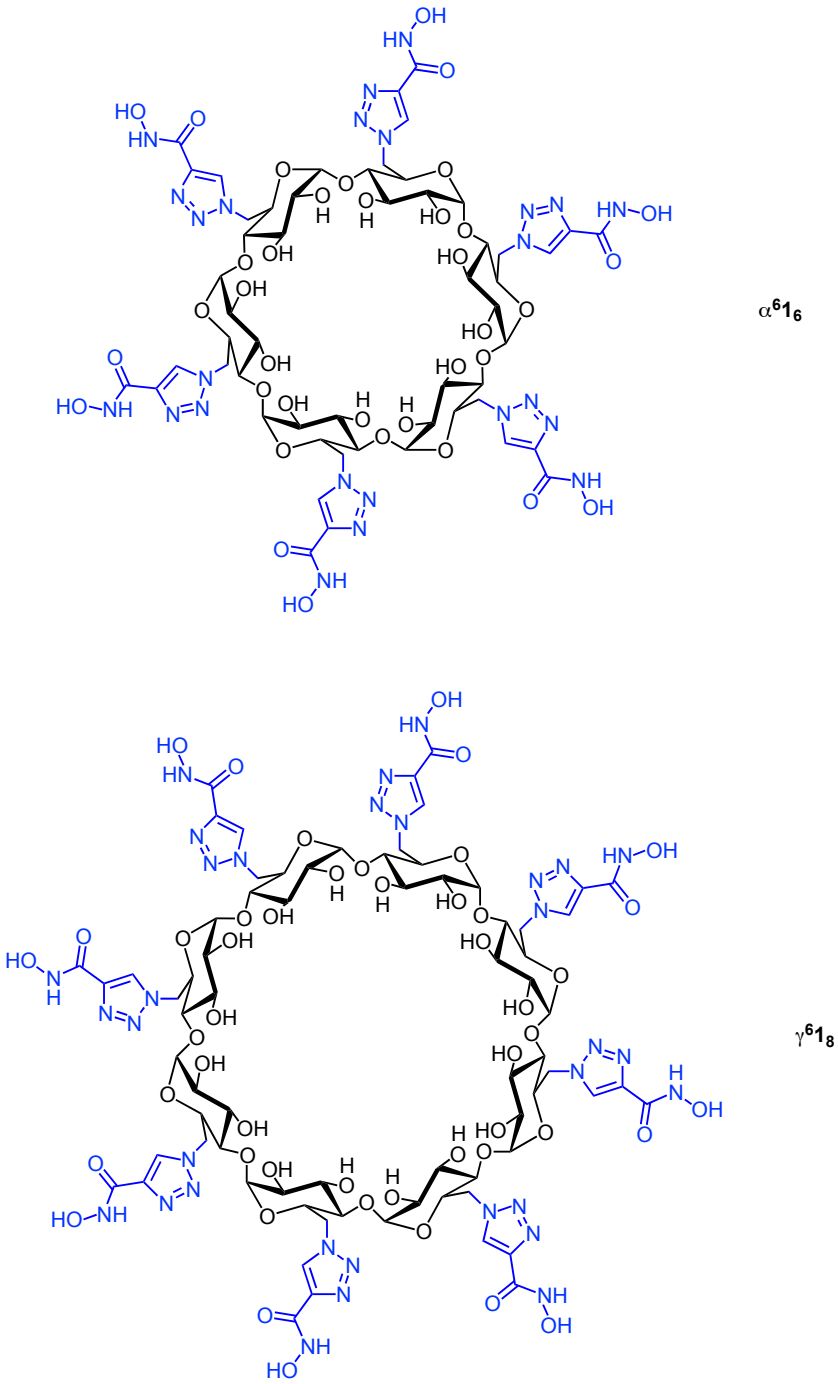


Figure S1: Structures of α- and γ-cyclodextrin derivatives **α^{6}1$_{6}$** and **γ^{6}1$_{8}$**.

2. Syntheses

All hydroxamic acid derivatives were obtained by coupling an alkyne derivative containing the hydroxamic acid group to known azides derived from α, β, or γ-cyclodextrin or 1-*O*-methyl-α-D-glucopyranose by using the copper(I)-catalyzed azide-alkyne cycloaddition, which regioselectively furnishes 1,4-disubstituted 1,2,3-triazoles.[1] The azides of the sugars were generally obtained by treating a derivative containing an appropriate leaving group in the correct position with sodium azide. 6-Azido-6-deoxy-β-cyclodextrin[2] was obtained from the corresponding tosylate.[3] The known biphenyl-4,4'-disulfonyl-A,D-capped β-cyclodextrin[4] served as starting material for 6^A,6^D-diazido-6^A,6^D-didesoxy-β-cyclodextrin.[5] A cyclodextrin with an azide group in 2-postion of one glucose unit was obtained from mono-2-(*p*-tolylsulfonyl)-β-cyclodextrin.[6] Since nucleophilic substitution proceeds under inversion of the stereogenic center this reaction afforded 2-azido-2-deoxy-*manno*-β-cyclodextrin.[7] Conversion of mono-2-(*p*-tolylsulfonyl)-β-cyclodextrin into β-cyclodextrin-2,3-*manno*-epoxide[8] and subsequent regioselective ring opening of the epoxide in the 3-position by using a known procedure afforded 3-azido-3-deoxy-*altro*-β-cyclodextrin.[9] The β-cyclodextrin derivative containing 7 azide groups in 6-position of the glucose units[10] was obtained from the corresponding β-cyclodextrin heptaiodide.[10] Analogously, the hexabromide of α-cyclodextrin[11] and the octabromide of γ-cyclodextrin[11] afforded the corresponding fully substituted azides of these cyclodextrins.[12] 6-Azido-6-deoxy-1-*O*-methyl-α-D-glucopyranose[13] was obtained by treating 6-(*p*-tolylsulfonyl)-6-deoxy-1-*O*-methyl-α-D-glucopyranose[13] with sodium azide.

General details. Products were characterised as follows: melting points, Müller SPM-X 300; NMR, Bruker Avance 600, Bruker DPX 400; MALDI-TOF-MS, Bruker Ultraflex TOF/TOF; ESI-MS, Bruker Esquire 3000; elemental analysis, Elementar vario Micro cube. All chemicals, unless other stated, are commercially available and used without further purification. For the preparative HPLC the following conditions were used: HPLC, Dionex UltiMate 3000; column, Thermo Fisher, BetaBasic-18, 250 × 21.2 mm, 5 µm particle size; flow, 12 mL/min; eluent, aqueous: 0.025 % aqueous ammonia, organic: acetonitrile; the following gradient was used for the isolation of the products: 0-6 min, 0 % organic; 6-22 min, linear increase to 15 % organic; 22-25 min, linear increase to 50 % organic; 25-30 min, 50 % organic; 30-37 min, linear decrease to 0 % organic; 37-45 min, 0 % organic.

General procedure for the syntheses of the hydroxamic acids. The respective carboxylic acid (350 mg) and the (substituted) hydroxylamine (1.1 equiv) were dissolved in dichloromethane (10 ml). A solution of DCC (1.1 equiv) in dichloromethane (10 ml) was added dropwise to the reaction mixture at 0 °C. The solution was allowed to warm to room temperature, and stirring was continued for 3 h. Afterwards, the precipitate was filtered off and the filtrate was evaporated to dryness. Purification of the products was achieved chromatographically on SiO_2 by using hexane/ethyl acetate mixtures.

***N*-Hydroxypent-4-ynamide.** Prepared from pentynoic acid and *O*-THP-protected hydroxylamine.[14] For the chromatographic purification hexane/ethyl acetate, 1:1 (*v/v*) was used. Yield 475 mg (89 %); R_f = 0.18 (hexane/ethyl acetate, 1:1 (*v/v*)); m.p. 97-98 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.59-1.80 (m, 8 H), 1.81 (s, 1 H), 2.35 (s, 2 H), 2.54-2.56 (m, 2 H), 4.96 (m, 1 H), 8.51 (s, 1 H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 14.9, 18.7, 25.1, 28.1, 29.8, 32.6, 62.7, 69.9, 102.8, 168.7; elemental analysis calcd (%) for $C_{10}H_{15}NO_3$: C 60.90, H 7.67, N 7.10; found C 60.90, H 7.43, N 7.16.

For deprotection the product was dissolved in methanol. After addition of *p*-toluenesulfonic acid monohydrate (20 mg) the reaction mixture was stirred for 18 h at 25 °C. After removal of the solvent, the residue was purified chromatographically on silica by using hexane/ethyl acetate, 1:3 (*v/v*) as eluent. Yield 188 mg (69 %); R_f = 0.30 (hexane/ethyl acetate, 1:3 (*v/v*)); m.p. 96-97 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.57 (s, 1 H), 2.42 (t, 2 H, *J*(H,H) = 6.9 Hz), 2.55-2.59 (m, 2 H), 7.35 (s, 1 H), 8.32 (s, 1 H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 14.1, 31.3, 71.5, 83.5, 167.2; elemental analysis calcd (%) for $C_5H_7NO_2$: C 53.09, H 6.24, N 12.38; found C 53.18, H 6.53, N 12.35.

***N*-Methoxypropiolamide.** Prepared from propiolic acid, methoxyammoniumchloride and *N*-ethyldiisopropylamine (1.1 equiv). For the chromatographic purification hexane/ethyl acetate, 1:1 (*v/v*) was used. The compound could not be obtained analytically pure. Yield 402 mg (46 %); R_f = 0.43 (hexane/ethyl acetate, 1:1 (*v/v*)); m.p. 75-76 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.60 (s, 3 H), 4.29 (s, 1 H), 11.87 (s, 1 H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 63.4, 75.6, 78.2, 149.0; ESI-MS (negative mode), *m/z* (%) 97.9 (100 %, M–H$^+$).

***N*-(Benzyloxy)-*N*-methylpropiolamide.** Prepared from propiolic acid and *N*-methyl-*O*-benzyl-hydroxylamine.[15] For the chromatographic purification hexane/ethyl acetate, 4:1 (*v/v*) was used. The product was obtained as an oil. Yield 766 mg (81 %); R_f = 0.15 (hexane/ethyl acetate, 4:1

(*v/v*)); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.17-3.19 (m, 4 H), 4.95 (s, 2 H), 7.37-7.44 (m, 5 H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 33.9, 75.5, 77.6, 79.2, 128.7, 129.1, 129.5, 134.1, 153.8; ESI-MS (positive mode), *m/z* (%) 211.8 (100 %, M+Na$^+$); elemental analysis calcd (%) for $C_{11}H_{11}NO_2$: C 69.83, H 5.86, N 7.40; found C 69.44, H 5.86, N 7.47.

General procedure for the copper(I)-catalyzed azide-alkyne cycloadditions. The respective azide (300 mg) was dissolved in an appropriate solvent mixture (10 ml) under an atmosphere of nitrogen. Copper(II)sulfate pentahydrate (0.01 equiv per azide group), sodium ascorbate (0.04 equiv per azide group), TBTA (0.01 equiv per azide group), and the alkyne (1.20 equiv per azide group) were added successively and the resulting mixture was stirred at 25 °C for 1 to 3 days. After conversion was complete, the reaction mixture was poured into acetone. The precipitate was filtered off and washed with acetone. Separation and purification of the product was achieved by preparative HPLC.

6-(4-(Hydroxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-β-cyclodextrin (β^{6}1). Prepared from 6-azido-6-deoxy-β-cyclodextrin[2] and *N*-hydroxypropiolamide[16] in ethanol/water, 1:1 (*v/v*). The reaction went to completion after 18 h. Yield: 147 mg (46 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.83-2.86 (m, 1 H), 3.06-3.09 (m, 1 H), 3.33-3.45 (s, 16 H, beneath H_2O signal), 3.56-3.77 (m, 22 H), 4.03-4.07 (m, 1 H), 4.51-4.63 (m, 6 H), 4.77-5.03 (m, 8 H), 5.75 (s, br, 14 H), 8.41 (s, 1 H), 9.06 (s, br, 1 H), 11.19 (s, br, 1 H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 50.6, 58.7-60.2, 69.8-73.2, 80.8-83.4, 101.2-102.2, 126.2, 141.7, 157.9; MALDI-TOF MS, *m/z* (%) 1251.5 (59 %, M–O+Na$^+$), 1267.6 (100 %, M+Na$^+$); elemental analysis calcd (%) for $C_{45}H_{72}N_4O_{36}\cdot 6\ H_2O\cdot C_3H_6O$: C 40.85, H 6.43, N 3.97; found C 40.66, H 6.69, N 4.22.

2-(4-(Hydroxycarbamoyl)-1*H*-1,2,3-triazole-1-yl)-2-deoxy-*manno*-β-cyclodextrin (β^{2}1). Prepared from 2-azido-2-deoxy-*manno*-β-cyclodextrin[7] and *N*-hydroxypropiolamide[16] in ethanol/water, 1:1 (*v/v*). The reaction went to completion after 3 d. Yield: 135 mg (39 %): ^{1}H NMR (D_2O, 400 MHz) δ 3.42-3.45 (m, 1 H), 3.51-3.73 (m, 11 H), 3.82-4.02 (m, 26 H), 4.24 (m, 1 H), 4.41-4.46 (m, 2 H), 4.79 (s, 1 H), 5.00-5.04 (m, 3 H), 5.09-5.13 (m, 3 H), 5.18-5.22 (m, 1 H), 8.62 (s, 1 H); ^{13}C NMR (D_2O, 101 MHz) δ 59.5-60.4, 62.3, 68.4, 70.9-73.2, 76.3, 77.6, 79.6-81.0, 100.4-101.7, 103.2, 127.5, 140.1, 159.4; MALDI-TOF MS, *m/z* (%) 1251.6 (100%, M–O+Na$^+$), 1267.6 (9%, M+Na$^+$); elemental analysis calcd (%) for $C_{45}H_{72}N_4O_{36}\cdot 4\ H_2O\cdot C_3H_6O$: C, 41.92; H, 6.30; N, 4.07 found C, 41.67; H, 6.04; N, 3.92.

3-(4-(Hydroxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-3-deoxy-*altro*-β-cyclodextrin (β^{3}1). Prepared from 3-azido-3-deoxy-*altro*-β-cyclodextrin[9] and *N*-hydroxypropiolamide[16] in ethanol/water, 1:1 (*v/v*). The reaction went to completion after 3 d. Yield: 180 mg, (56 %): ^{1}H NMR (D_2O, 400 MHz) δ 3.44-3.48 (m, 1 H), 3.53-3.75 (m, 11 H), 3.81-4.04 (m, 26 H), 4.27 (m, 1 H), 4.44-4.48 (m, 2 H), 4.79 (s, 1H), 5.02-5.06 (m, 3 H), 5.11-5.15 (m, 3 H), 5.22-5.25 (m, 1 H), 8.68 (s, 1 H); ^{13}C NMR (D_2O, 101 MHz) δ 59.5, 60.3-60.4, 62.3, 68.4, 70.9-73.2, 76.3, 77.6, 79.6-81.0, 100.4-101.8, 103.2, 127.8, 139.8, 159.7; MALDI-TOF MS, *m/z* (%) 1251.6 (100%, M–O+Na^+), 1267.6 (8%, M+Na^+); elemental analysis calcd (%) for $C_{45}H_{72}N_4O_{36}$·4 H_2O·C_3H_6O: C, 41.92; H, 6.30; N, 4.07 found C, 41.65; H, 6.25; N, 3.97.

Bis-6^A,6^D-(4-(hydroxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6^A,6^D-didesoxy-β-cyclodextrin (β^{6}1$_2$). Prepared from 6^A,6^D-diazido-6^A,6^D-didesoxy-β-cyclodextrin[5] and N-hydroxypropiolamide[16] in ethanol/water, 1:1 (*v/v*). The reaction went to completion after 3 d. Yield 64.5 mg (19 %); ^{1}H NMR (D_2O, 600 MHz) δ 2.85-2.92 (m, 2 H), 3.11-3.16 (m, 2 H), 3.45-3.76 (m, 19 H), 3.85-4.09 (m, 15 H), 4.27-4.40 (m, 2 H), 4.96-5.24 (m, 7 H), 5.23 (d, 2 H, *J*(H,H) = 3.5 Hz), 8.45-8.47 (2 × s, 2 H); ^{13}C NMR (D_2O, 151 MHz) δ 51.4-51.6, 59.0-60.6, 69.8-73.0, 80.1-82.6, 100.7-102.0, 127.8-128.0, 139.9-140.0, 159.3; MALDI-TOF MS, *m/z* (%) 1345.7 (38 %, M–2O+Na^+), 1361.7 (100 %, M–O+Na^+), 1377.7 (97 %, M+Na^+), 1393.7 (35 %, M+K^+); elemental analysis calcd (%) for $C_{48}H_{74}N_8O_{37}$·8 H_2O·C_3H_6O: C 39.33, H 6.21, N 7.20; found C 39.57, H 6.49, N 7.17.

Heptakis-6-(4-(hydroxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6-heptadesoxy-β-cyclodextrin (β^{6}1$_7$). Prepared from heptakis-6-azido-6-heptadesoxy-β-cyclodextrin[10] and *N*-hydroxypropiolamide[16] in DMSO/water, 4:1 (*v/v*). The reaction went to completion after 3 d. Purification of the product did not require HPLC, but was achieved by recrystallization from ethanol/aqueous ammonia. Yield 293 mg (67 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.23-3.70 (m, 21 H), 4.10 (s, 7 H), 4.54 (s, 14 H), 5.10 (s, 7 H), 5.88 (s, 14 H), 8.39 (s, 7 H), 9.00 (s, 7 H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 49.6, 69.1-69.4, 71.6-71.9, 72.5-72.7, 82.1-82.4, 101.3-102.0, 127.8, 141.2, 157.7; MALDI-TOF MS, *m/z* (%) 1874.4 (11 %, M–2O+H^+), 1890.4 (46 %, M–O+H^+), 1896.4 (46 %, M–2O+Na^+), 1906.4 (59 %, M+H^+), 1912.4 (46 %, M–O+Na^+), 1928.5 (100 %, M+Na^+); elemental analysis calcd (%) for $C_{63}H_{84}N_{28}O_{42}$·7 H_2O·2 C_2H_6O: C 37.89, H 5.22, N 18.47; found C 37.81, H 5.20, N 18.52.

Hexakis-6-(4-(hydroxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6-hexadesoxy-α-cyclodextrin (α^{6}1$_6$). Prepared from hexakis-6-azido-6-hexadesoxy-α-cyclodextrin[12] and *N*-hydroxypropiolamide[16] in

DMSO/water, 4:1 (*v/v*). The reaction went to completion after 4 d. Purification of the product did not require HPLC, but was achieved by recrystallisation from ethanol/aqueous ammonia. Yield 328 mg (75 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.18-3.23 (m, 12 H), 3.82-3.86 (m, 6 H), 4.23-4.25 (m, 6 H), 4.56-4.65 (m, 12 H), 5.07 (s, 6 H), 5.49-5.59 (m, 12 H), 8.39 (s, 6 H), 9.08 (s, 6 H), 11.10 (s, 6 H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 49.8, 69.4, 71.2, 72.5, 82.7, 101.5, 127.6, 141.2, 157.7; MALDI-TOF MS, *m/z* (%) 1601.8 (81 %, M–2O+H$^+$), 1617.8 (100 %, M–O+H$^+$), 1623.8 (22 %, M–2O+Na$^+$), 1633.8 (41 %, M+H$^+$), 1639.8 (35 %, M–2O+K$^+$), 1655.8 (37 %, M+Na$^+$), 1671.9 (32 %, M+K$^+$); elemental analysis calcd (%) for $C_{54}H_{72}N_{24}O_{36}\cdot 7\ H_2O\cdot 0.5\ C_2H_6O$: C 37.06, H 5.03, N 18.86; found C 37.19, H 5.09, N 18.72.

Octakis-6-(4-(hydroxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6-octadesoxy-γ-cyclodextrin (γ^{6}1$_8$). Prepared from octakis-6-azido-6-octadesoxy-γ-cyclodextrin[12] and *N*-hydroxypropiolamide[16] in DMSO/water, 4:1 (*v/v*). The reaction went to completion after 4 d. Purification of the product did not require HPLC, but was achieved by recrystallisation from ethanol/aqueous ammonia. Yield 293 mg (67 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.19-3.24 (m, 16 H), 3.65-3.70 (m, 8 H), 4.10-4.12 (m, 8 H), 4.44-4.55 (m, 16 H), 5.15 (s, 8 H), 5.91 (m, 16 H), 8.40 (s, 8 H), 9.18 (s, 8 H), 11.00 (s, 8 H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 49.6, 69.2, 72.0, 72.4, 81.8, 101.5, 127.7, 141.1, 157.7; MALDI-TOF MS, *m/z* (%) 2167.7 (100 %, M–2O+Na$^+$), 2183.7 (100 %, M–O+Na$^+$), 2200.0 (42 %, M+Na$^+$); elemental analysis calcd (%) for $C_{72}H_{96}N_{32}O_{48}\cdot 5\ H_2O\cdot C_3H_6O$: C 38.73, H 4.85, N 19.27; found C 38.89, H 4.86, N 18.99.

6-(4-(2-(Hydroxycarbamoyl)-ethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-β-cyclodextrin (β^{6}2). Prepared from 6-azido-6-deoxy-β-cyclodextrin[2] and *N*-hydroxypent-4-ynamide in ethanol/water, 1:1 (*v/v*). The reaction went to completion after 18 h. Yield 90.0 mg (27 %); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (t, 2 H, *J*(H,H) = 7.7 Hz), 2.82 (t, 2 H, *J*(H,H) = 7.5 Hz), 3.31-3.38 (m, 21 H), 3.55-3.68 (m, 21 H), 4.48-4.56 (m, 6 H), 4.83 (s, 7 H), 5.65 5.88 (m, 14 H), 7.76 (s, 1 H), 8.74 (s, 1 H), 10.45 (s, 1 H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 21.2, 31.7, 50.4, 58.8, 70.0-73.2, 80.8-83.5, 101.2-102.2, 122.8, 145.6, 168.3; MALDI-TOF MS, *m/z* (%) 1273.5 (2 %, M+H$^+$), 1280.6 (61 %, M–O+Na$^+$), 1295.7 (100 %, M+Na$^+$), 1311.7 (20 %, M+K$^+$); elemental analysis calcd (%) for $C_{47}H_{76}N_4O_{36}\cdot 3\ H_2O$: C 42.53, H 6.23, N 4.22; found C 42.47, H 6.32, N 4.44.

6-(4-(*N*-Methyl-methoxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-β-cyclodextrin (β^{6}3). Prepared from 6-azido-6-deoxy-β-cyclodextrin[2] and *N*-methoxy-*N*-methylpropiolamide[17] in

ethanol/water, 1:1 (*v/v*). The reaction went to completion after 18 h. Yield 78 mg (24 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.83-2.87 (m, 1 H), 3.04-3.06 (m, 1 H), 3.24-3.42 (m, 15 H), 3.49-3.82 (m, 29 H), 4.08-4.13 (m, 1 H), 4.29-4.32 (m, 1 H), 4.49-4.67 (m, 6 H), 4.75-5.03 (m, 7 H), 5.64-5.91 (m, 14 H), 8.54 (s, 1 H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 32.7, 50.6, 58.8-60.3, 61.3, 69.8-73.3, 80.6-83.6, 101.2-102.3, 129.0, 139.8, 160.4; MALDI-TOF MS, *m/z* (%) 1265.8 (14 %, M–OMe+H$^+$), 1295.8 (100 %, M+Na$^+$), 1311.8 (23 %, M+K$^+$); elemental analysis calcd (%) for $C_{47}H_{76}N_4O_{36}\cdot H_2O\cdot 0.5\ C_3H_6O$: C 44.13, H 6.18, N 4.24; found C 44.09, H 5.98, N 4.05.

6-(4-(Methoxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-β-cyclodextrin (β^{6}4). Prepared from 6-azido-6-deoxy-β-cyclodextrin[2] and *N*-methoxypropiolamide in ethanol/water, 1:1 (*v/v*). The reaction went to completion after 18 h. Yield 74 mg (23 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.80 (d, 1 H, *J*(H,H) = 10.6 Hz), 3.04 (d, 1 H, *J*(H,H) = 11.2 Hz), 3.30-3.41 (m, 12 H), 3.55-3.67 (m, 29 H), 4.07 (t, 1 H, *J*(H,H) = 9.8 Hz), 4.38 (s, 1 H), 4.50-4.65 (m, 6 H), 4.73-5.02 (m, 7 H), 5.62-5.95 (m, 14 H), 8.56 (s, 1 H), 11.91 (s, 1 H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 50.7, 58.7-60.4, 63.3, 69.9-73.3, 80.7-83.5, 101.1-102.3, 127.2, 141.1, 157.5; MALDI-TOF MS, *m/z* (%) 1251.5 (42 %, M–OMe+H$^+$), 1267.4 (5 %, M–Me+H$^+$), 1281.5 (100 %, M+Na$^+$), 1297.5 (15 %, M+K$^+$); elemental analysis calcd (%) for $C_{46}H_{74}N_4O_{36}\cdot H_2O$: C 43.26, H 6.00, N 4.39; found C 43.02, H 5.99, N 4.17.

6-(4-(*N*-Methyl-hydroxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-β-cyclodextrin (β^{6}5). Prepared from 6-azido-6-deoxy-β-cyclodextrin[2] and *N*-(benzyloxy)-*N*-methylpropiolamide in ethanol/water, 1:1 (*v/v*). The reaction went to completion after 18 h. The crude product was dissolved in methanol/water, 1:2 (*v/v*) (15 mL). Palladium on charcoal (40 mg) was added and the reaction mixture was stirred for 18 h at 25 °C under an atmosphere of hydrogen. After filtration through Celite purification was achieved by HPLC as described above. Yield 105 mg (32 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.87-2.89 (m, 1 H), 3.07-3.10 (m, 1 H), 3.30-3.38 (m, 15 H), 3.55-3.66 (m, 26 H), 3.76-3.79 (m, 1 H), 4.05-4.09 (m, 1 H), 4.50-4.68 (m, 6 H), 4.75-5.04 (m, 7 H), 5.66-5.93 (m, 14 H), 8.49 (s, 1 H), 10.09 (s, 1 H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 36.5, 50.4, 58.9-60.3, 69.8-73.3, 80.8-83.5, 101.2-102.3, 128.7, 139.9, 160.4; MALDI-TOF MS, *m/z* (%) 1259.6 (1 %, M+H$^+$), 1265.7 (15 %, M–O+H$^+$), 1281.7 (100 %, M+Na$^+$), 1297.7 (11 %, M+K$^+$); elemental analysis calcd (%) for $C_{46}H_{74}N_4O_{36}\cdot 2\ H_2O$: C 42.66, H 6.07, N 4.33; found C 42.70, H 6.15, N 4.26.

Bis-6^{A},6^{D}-(4-(*N*-methylhydroxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6^{A},6^{D}-dideoxy-β-cyclodextrin (β^{6}5$_{2}$). Prepared from 6^{A},6^{D}-diazido-6^{A},6^{D}-didesoxy-β-cyclodextrin[5] and *N*-(benzyloxy)-*N*-methylpropiolamide in ethanol/water, 1:1 (*v/v*). The reaction went to completion after 2 d. The crude product was dissolved in methanol/water, 1:2 (*v/v*) (15 mL). Palladium on charcoal (40 mg) was added and the reaction mixture was stirred for 18 h at 25 °C under an atmosphere of hydrogen. After filtration through Celite purification was achieved by HPLC as described above. Yield 57.3 mg (16 %); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 2.98-3.39 (m, 20 H), 3.54-3.76 (m, 24 H), 4.02-4.07 (m, 2 H), 4.49 (s, 2 H), 4.50-4.68 (m, 5 H), 4.70-5.05 (m, 7 H), 5.70-5.93 (m, 14 H), 8.46 (s, 2 H), 10.22 (s, 2 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ 36.4, 50.2, 59.1-60.3, 69.7-73.3, 80.9-83.3, 101.4-102.3, 129.0, 139.7, 160.3; MALDI-TOF MS, *m/z* (%) 1373.7 (14 %, M–O–Me+Na^{+}), 1389.8 (100%, M–O+Na^{+}), 1405.8 (79 %, M+Na^{+}); elemental analysis calcd (%) for $C_{50}H_{78}N_8O_{37}\cdot 3\ H_2O\cdot C_3H_6O$: C 42.57, H 6.07, N 7.49; found C 42.41, H 5.99, N 7.20.

Heptakis-6-(4-(*N*-Methylhydroxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6-heptadesoxy-β-cyclodextrin (β^{6}5$_{7}$). Prepared from heptakis-6-azido-6-heptadesoxy-β-cyclodextrin[10] and *N*-(benzyloxy)-*N*-methylpropiolamide in DMSO/water, 4:1 (*v/v*). The reaction went to completion after 4 d. The crude product was dissolved in methanol/water, 1:2 (*v/v*) (20 mL). Palladium on charcoal (40 mg) was added and the reaction mixture was stirred for 3 d at 25 °C under an atmosphere of hydrogen. After filtration through Celite the product was precipitated by pouring the filtrate into acetone. After drying it turned out to be analytically pure. Yield 223 mg (49 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.17-3.26 (m, 35 H), 3.69-374 (m, 7 H), 4.11-4.13 (m, 7 H), 4.42-4.53 (m, 14 H), 5.01 (m, 7 H), 5.90-6.02 (m, 14 H), 8.34 (s, 7 H), 10.29 (s, 7 H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 36.3, 49.5, 69.5, 71.7, 72.4, 82.4, 101.6, 129.4, 139.5, 159.9; MALDI-TOF MS, *m/z* (%) 1994.0 (28 %, M–O–Me+Na^{+}), 2010.1 (96 %, M–O+Na^{+}), 2026.1 (100 %, M+Na^{+}); elemental analysis calcd (%) for $C_{70}H_{98}N_{28}O_{42}\cdot 5\ H_2O\cdot C_3H_6O$: C 41.31, H 5.47, N 17.75; found C 41.54, H 5.48, N 17.56.

6-(4-(Hydroxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-1-*O*-methyl-α-D-glucopyranose (G^{6}1). Prepared from 6-azido-6-deoxy-1-*O*-methyl-α-D-glucopyranose[13] and *N*-hydroxypropiolamide[16] in ethanol/water, 1:1 (*v/v*). The reaction went to completion after 18 h. Yield: 182 mg (44 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.95-3.02 (m, 4 H), 3.18-3.19 (m, 1 H), 3.37-3.41 (m, 1 H), 3.69-3.73 (m, 1 H), 4.43-4.50 (m, 2 H), 4.73-4.77 (m, 1 H), 4.85 (s, 1 H), 4.98 (s, 1 H), 5.41 (s, 1 H), 8.42 (s, 1 H), 9.24 (br, 1 H), 10.95 (s, 1 H); ^{13}C NMR (DMSO-d_6, 101 MHz)

δ 51.1, 54.2, 70.2, 71.6, 71.7, 73.1, 99.8, 127.1, 141.0, 157.7; MALDI-TOF MS, *m/z* (%) 289.0 (5 %, M–O+H^+), 305.0 (39 %, M+H^+), 311.0 (40 %, M–O+Na^+), 327.0 (M+Na^+); elemental analysis calcd (%) for $C_{10}H_{16}N_4O_7$: C 39.48, H 5.30, N 18.41; found C 39.15, H 5.33, N 18.21.

6-(4-(*N*-Methylhydroxycarbamoyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-1-*O*-methyl-α-D-glucopyranose (G^{6}5). Prepared from 6-azido-6-deoxy-1-*O*-methyl-α-D-glucopyranose[13] and *N*-(benzyloxy)-*N*-methylpropiolamide in ethanol/water, 1:1 (*v/v*). The reaction went to completion after 18 h. Afterwards, the solvent was evaporated and the residue was dissolved in methanol/water, 1:2 (v/v) (15 mL). Palladium on charcoal (40 mg) was added and the reaction mixture was stirred for 18 h at 25 °C under an atmosphere of hydrogen. After filtration through Celite purification was achieved chromatographically on silica by using methanol/dichloromethane, 1:5 as eluent. Yield 137 mg (47 %); R_f = 0.17 (methanol/ dichloromethane 1:5 (*v/v*)); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 2.96-3.02 (m, 4 H), 3.17 (s, 1 H), 3.20 (s, 3 H), 3.38-3.42 (m, 1 H), 3.70-3.73 (m, 1 H), 4.46-4.50 (m, 2 H), 4.74-4.76 (m, 1 H), 4.85 (d, 1 H, *J*(H,H) = 6.3 Hz), 4.98 (d, 1 H, *J*(H,H) = 4.6 Hz), 5.40 (d, 1 H, *J*(H,H) = 5.6 Hz), 8.40 (s, 1 H), 10.30 (s, 1 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ 36.4, 50.9, 54.3, 70.2, 71.7, 71.7, 73.1, 99.8, 128.8, 139.4, 160.3; MALDI-TOF MS, *m/z* (%) 303.0 (9 %, M–O+H^+), 319.0 (31 %, M+H^+), 325.0 (25 %, M–O+Na^+), 341.0 (100 %, M+Na^+); elemental analysis calcd (%) for $C_{11}H_{18}N_4O_7 \cdot 0.5\ H_2O$: C 40.37, H 5.85, N 17.12; found C 40.10, H 5.67, N 17.00.

3. Enzymatic Assays

Materials. Acetylthiocholine iodide (ATCh), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), and tris(hydroxymethyl)aminomethane (TRIS) were obtained from Sigma-Aldrich (Taufkirchen, Germany). Disodium hydrogenphosphate dihydrate, potassium dihydrogenphosphate, and hydrochloric acid were purchased from Roth (Karlsruhe, Germany). Chloroform (SupraSolve) was obtained from Merck (Darmstadt, Germany), ammonia (6.0) from Linde (Munich, Germany), and helium (6.0) from Air Liquide Germany (Munich, Germany). Ethyl dimethylphosphoramido-cyanidate (tabun; GA; >98% by ^{1}H NMR and ^{31}P NMR) and the internal standard (IS) propyl dimethylphosphoramidocyanidate (>97% by ^{1}H NMR and ^{31}P NMR) were made available by the German Ministry of Defence (all experiments with GA were performed at the Bundeswehr Institute for Pharmacology and Toxicology in Munich). Hemoglobin-free human erythrocyte ghosts served as human AChE (hAChE) source and were prepared according to Dodge *et al.*[18] with minor modifications.[19] AChE activity was adjusted to 4000 U/L by dilution with sodium phosphate buffer (0.1 M, pH 7.40). Aliquots of ghosts were stored at –80 °C. Prior to use, ghosts were homogenised with a Sonoplus HD 2070 ultrasonic homogenator (Bandelin electronic, Berlin, Germany) twice for 5 s with a 20 s interval to achieve a homogeneous matrix.

Qualitative Enzymatic Assay. This assay was performed by using a Tecan Freedom Evo liquid handling system (Männedorf, Switzerland) analogously to the previously reported procedure.[20] Specifically, solutions of a respective cyclodextrin or glucose derivative (40 µL, 2 mM, 0.1 M TRIS-HCl buffer) and GA (40 µL, 36 µM, 0.1 M TRIS-HCl buffer) were added to TRIS-HCl buffer (80 µL, 0.1 M, pH 7.40) and incubated at 37.0 °C. Immediately after mixing, a sample (25 µL) was transferred to a 24-well microplate filled with pre-heated buffer (2.40 mL, 0.1 M TRIS-HCl buffer, pH 7.40 and 0.3 mM DTNB at 37.0 °C) and hAChE (10 µL). After the addition of ATCh (50 µL, 49.7 mM) the microplate was transferred to a photometer and maintained at 37.0 °C. AChE activity was determined by a modified Ellman assay[21,22,23] by following the change in absorption at 436 nm for 30 min to determine the rate of the formation of the 2-nitro-5-thiobenzoate dianion (Ellman assay). Further aliquots of the incubation mixture were taken after 30 min and 60 min and treated analogously.

First order inhibition rate constants k_1 were determined by non-linear regression analysis of the obtained curves by using Prism 5.0 (GraphPad Software, San Diego, CA, USA).[24] Rate constants

k_1^{ref} were determined by performing the assay in the absence of cyclodextrin. Finally, an independent experiment performed in the absence of both cyclodextrin and organophosphate furnished the rate constant k_1^{native}. Relating k_1 to k_1^{ref} and k_1^{native} according to $(k_1^{ref} - k_1)/(k_1^{ref} - k_1^{native}) \cdot 100$ % yielded Δk_1, a term describing the activity of the cyclodextrin.[25] If the extent of enzyme inhibition is the same in the absence and the presence of the cyclodextrin ($k_1^{ref} = k_1$) the cyclodextrin is inactive and $\Delta k_1 = 0$ %. If, however, the organophosphate disappears faster in the presence of the cyclodextrin than during the background reaction, k_1 becomes smaller with respect to k_1^{ref} until it approaches the value of k_1^{native}. As a consequence, Δk_1 increases up to 100 % for full enzymatic activity ($k_1 = k_1^{native}$). All results are means of n = 3 experiments. Standard deviation of a series of identical measurements with the same batch of AChE is <10%. Because the results can vary more strongly if different enzyme preparations are used we assume an overall error of 20%. For the flowchart of this assay, see Figure S2.

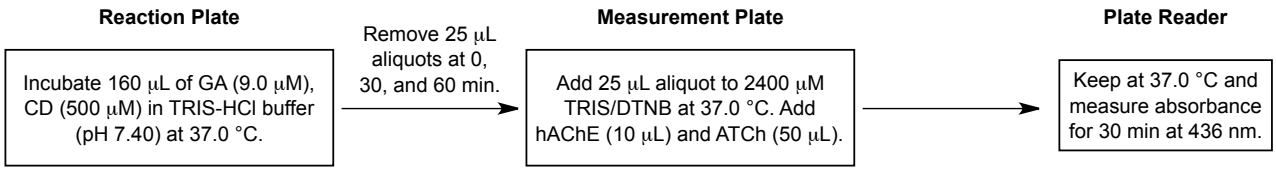


Figure S2: Flowchart showing the protocol of the qualitative enzymatic assay.

Quantitative Enzymatic Assay. The quantitative enzymatic assay was performed as described before.[26] In brief, GA (1 µM) was incubated with a respective cyclodextrin or glucose derivative (500 µM) in TRIS-HCl buffer (0.1 M, pH 7.40) at 37.0 °C. Samples (10 µL) were taken at defined times, diluted with TRIS-HCl buffer (16 µL), and 10 µL of the diluted solutions were incubated with a nine-fold excess of hemoglobin-free erythrocyte ghosts for 30 min at 37.0 °C. An aliquot was taken and residual AChE activity was measured photometrically (Cary 50 Bio, Varian GmbH, Darmstadt, Germany) by using a modified Ellman assay.[22,23] Activities thus obtained were referenced to a control and data are given as % of control. Processing of experimental data for the determination of detoxification rate was performed by non-linear regression analysis using curve-fitting routines implemented in GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Results shown are means of n = 2 experiments. Figure S3 shows a flowchart of this assay.

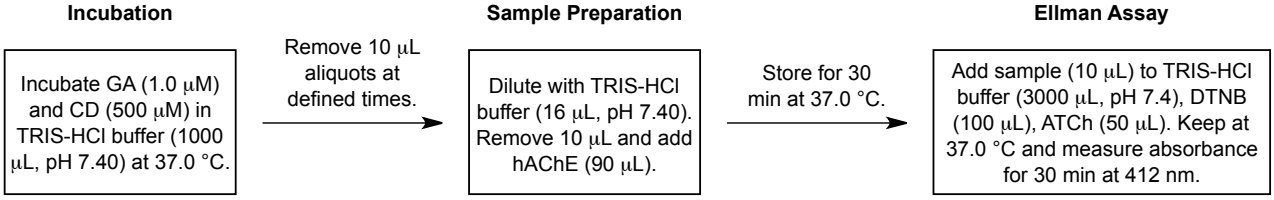


Figure S3: Flowchart showing the protocol of the quantitative enzymatic assay.

Figure S4 -Figure S16 show the time-dependent decrease of GA concentration (initial concentration 1 μM) mediated by the different hydroxamic acids (0.5 mM) at pH 7.40 and 37.0 °C monitored using the quantitative enzymatic assay (means of n = 2). The data points show the time-dependent decrease of AChE inhibition resulting from the detoxification of racemic GA, which correlates with GA concentration. The obtained values were referenced to a control activity. Kinetic parameters can be calculated from the results if degradation of the two GA enantiomers proceeds with the same rate. The lines show the curve fits of the measurements to a first-order rate reaction ($R^2 \geq 0.98$).

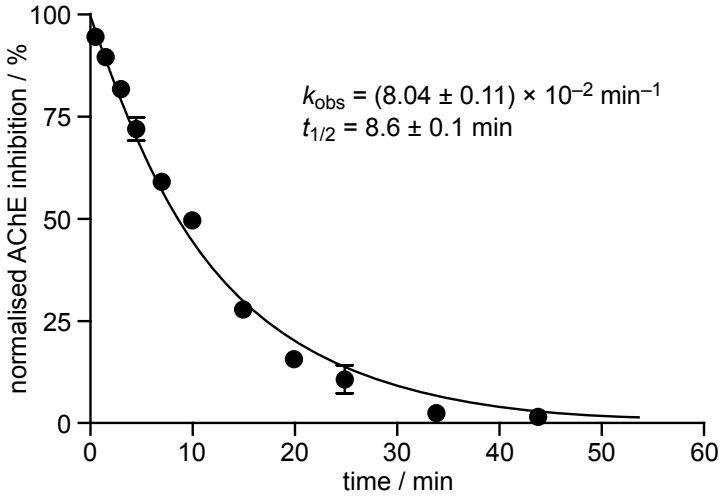


Figure S4: Hydroxamic acid **β^{6}1**.

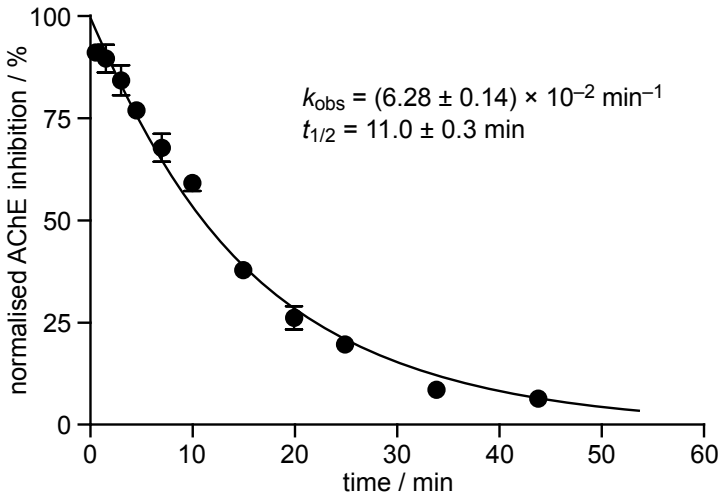


Figure S5: Hydroxamic acid **β²1**.

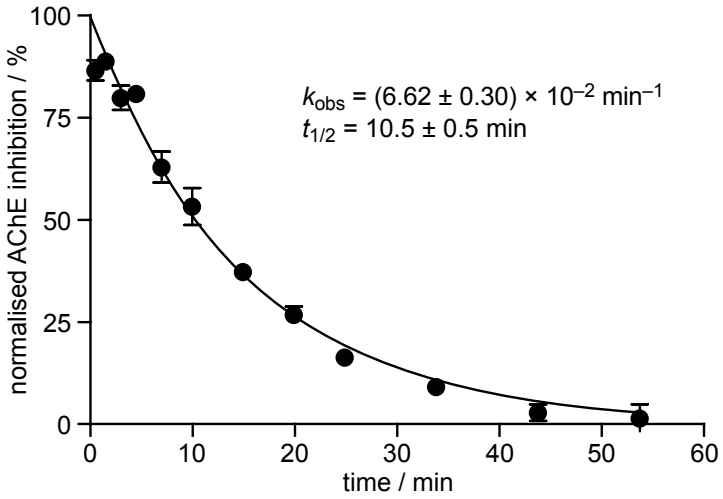


Figure S6: Hydroxamic acid **β³1**.

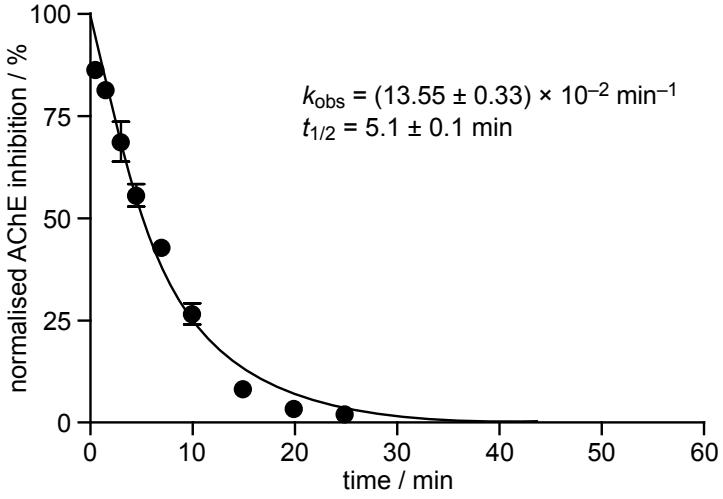


Figure S7: Hydroxamic acid **β⁶1₂**.

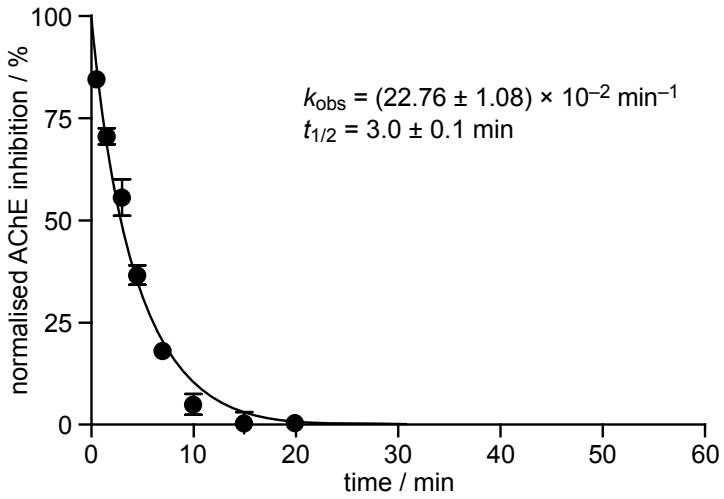


Figure S8: Hydroxamic acid $\boldsymbol{\beta^6 1_7}$.

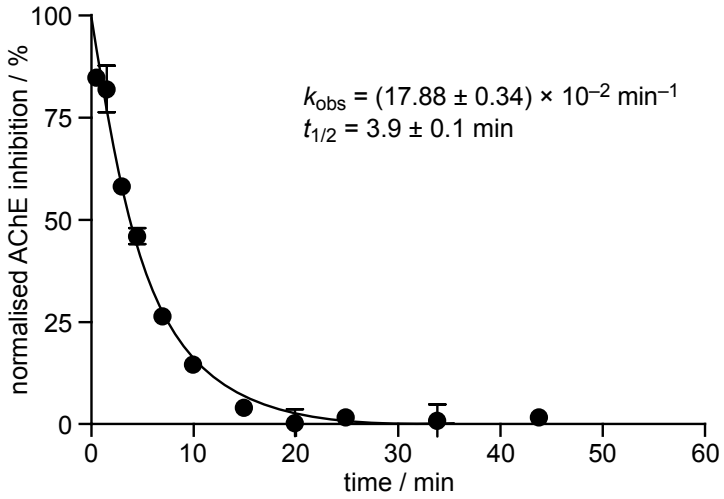


Figure S9: Hydroxamic acid $\boldsymbol{\alpha^6 1_6}$.

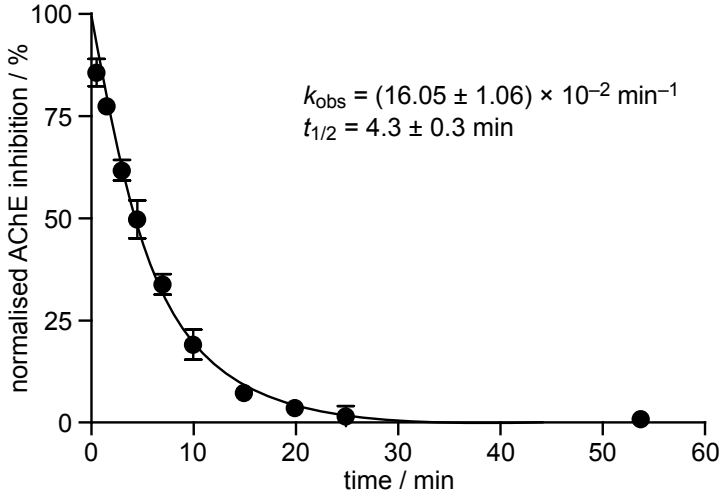


Figure S10: Hydroxamic acid $\boldsymbol{\gamma^6 1_8}$.

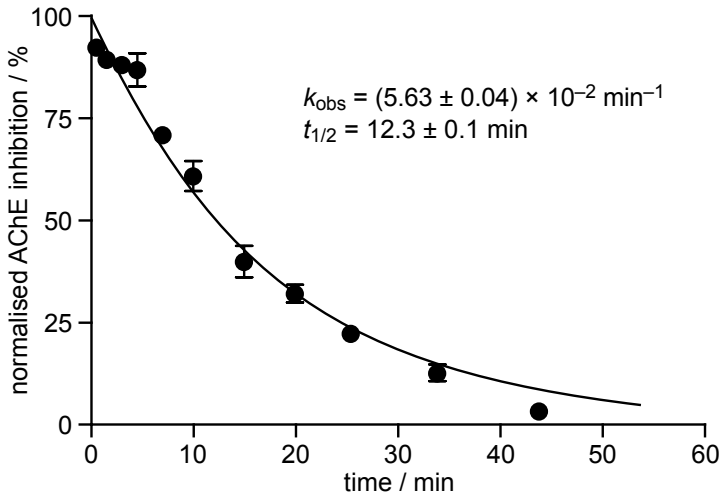


Figure S11: Hydroxamic acid **β^{6}5**.

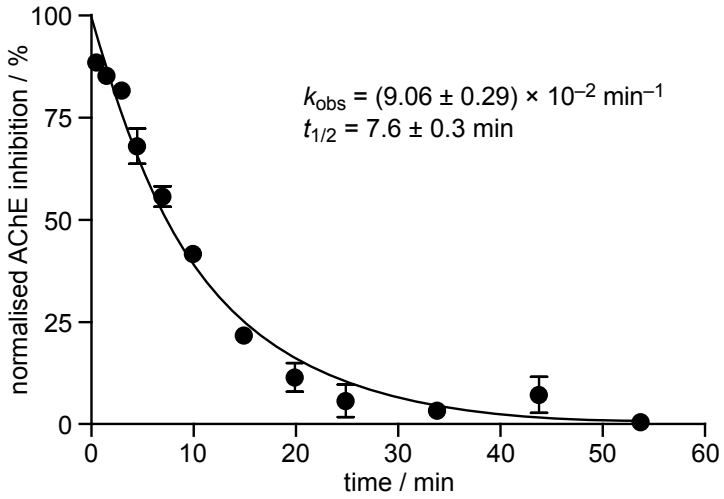


Figure S12: Hydroxamic acid **β^{6}5$_7$**.

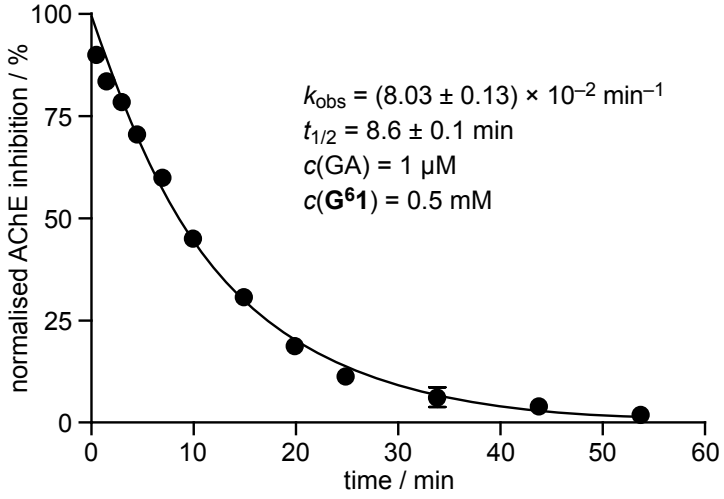


Figure S13: Hydroxamic acid **G^{6}1** (scavenger concentration 0.5 mM).

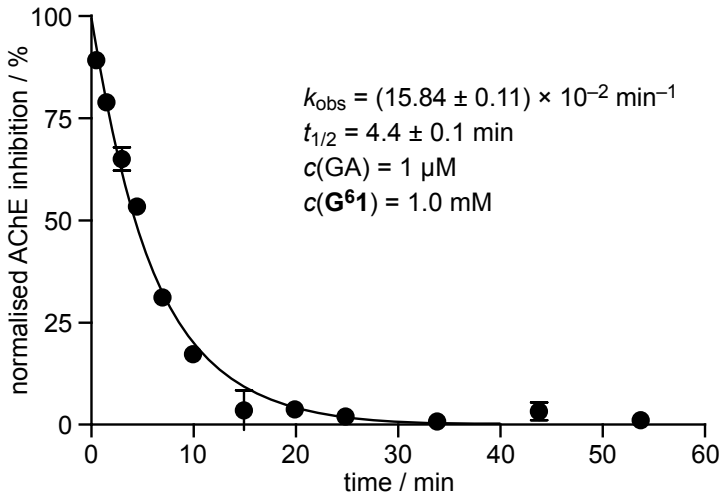


Figure S14: Hydroxamic acid **G^{6}1** (scavenger concentration 1.0 mM).

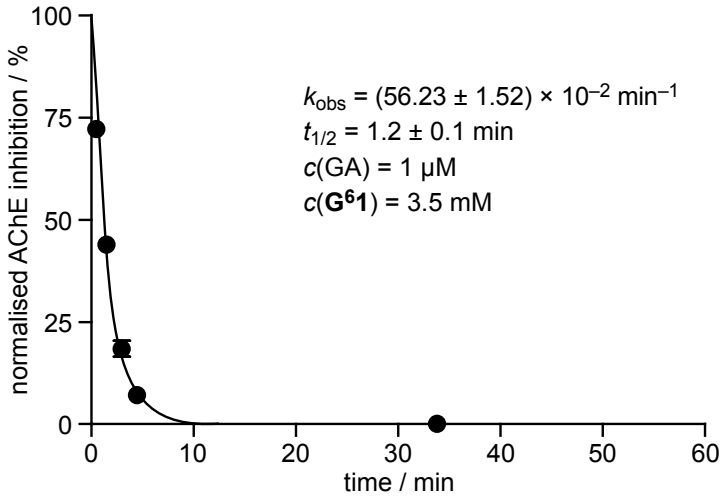


Figure S15: Hydroxamic acid **G^{6}1** (scavenger concentration 3.5 mM).

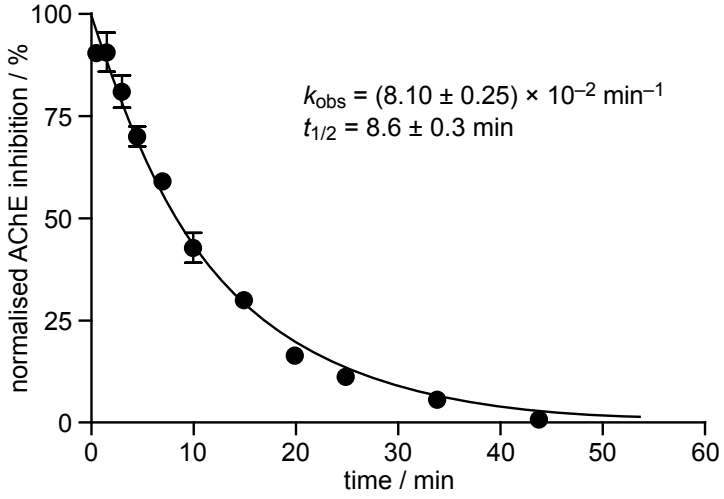


Figure S16: Hydroxamic acid **G^{6}5**.

Repeated Incubation of Cyclodextrins with Tabun. This assay has been described previously.[27] A stock solution of a hydroxamic acid was prepared in TRIS-HCl buffer (0.1 M, pH 7.40) and divided into a processing aliquot (990 µL) and a control aliquot (298 µL). The resulting solutions were stored at 37.0 °C. A solution of GA in acetonitrile (10.0 µL, 1.00 mM) was added to the processing aliquot to obtain an overall volume of 1000 µL and final concentrations of 10.0 µM GA and 150.0 µM scavenger (Figure S17). Aliquots (25 µL) were removed at defined times and first order inhibition rate constants k_1 were determined by using the qualitative enzymatic assay (see above). Degradation of GA was complete after 6 h. Afterwards, a solution of GA in acetonitrile (34.0 µL, 6.17 mM) was added to 699 µL of the remaining sample solution to obtain concentrations of 143 µM hydroxamic acid and 286 µM GA. This solution was incubated at 37.0 °C overnight. On the next day the Ellman Assay was performed again. A control sample without cyclodextrin, which allows assessing spontaneous GA hydrolysis, was analogously processed on the first day. For comparison, k_1 was also determined on the second day of the control aliquot. Prior to this measurement, 18 µL of TRIS-HCl buffer was added to the control aliquot to take the dilution of the processed sample resulting from the GA addition into account. The results obtained for compounds **β⁶1** and **β⁶5** are shown in, respectively, Figure S18 and Figure S19.

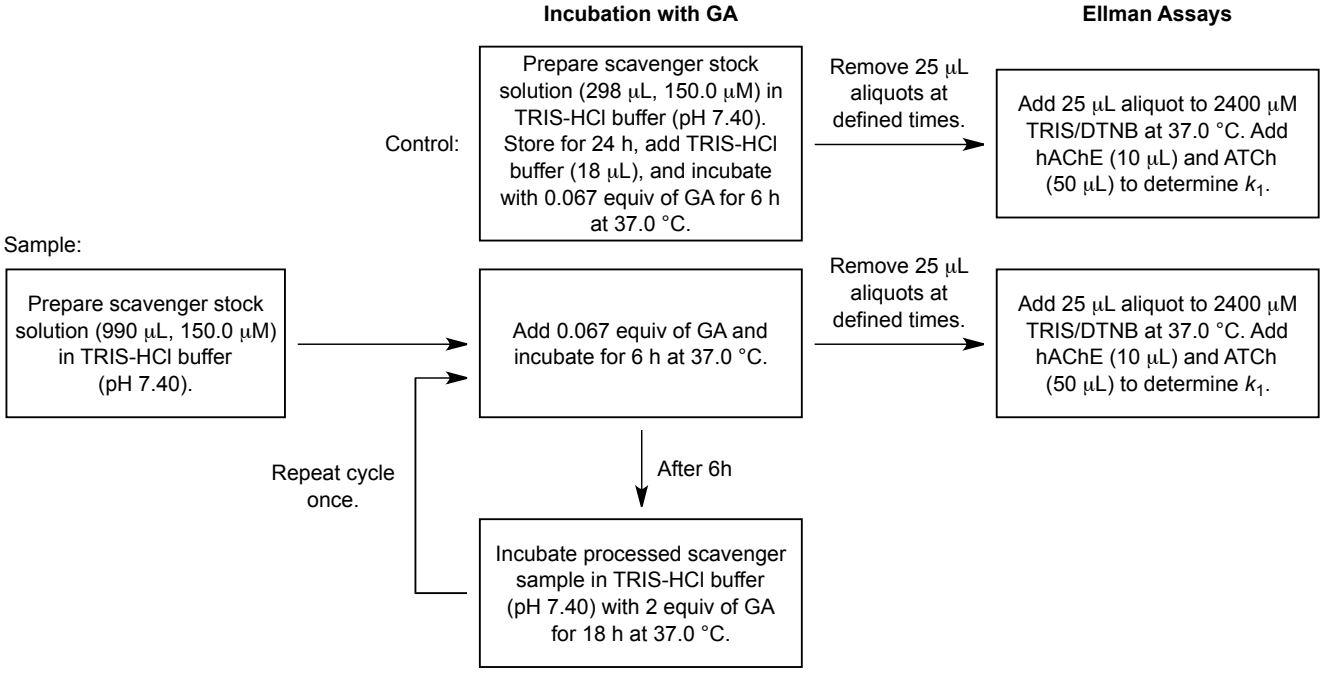


Figure S17: Flowchart showing the protocol of the repeated incubation of cyclodextrin with GA.

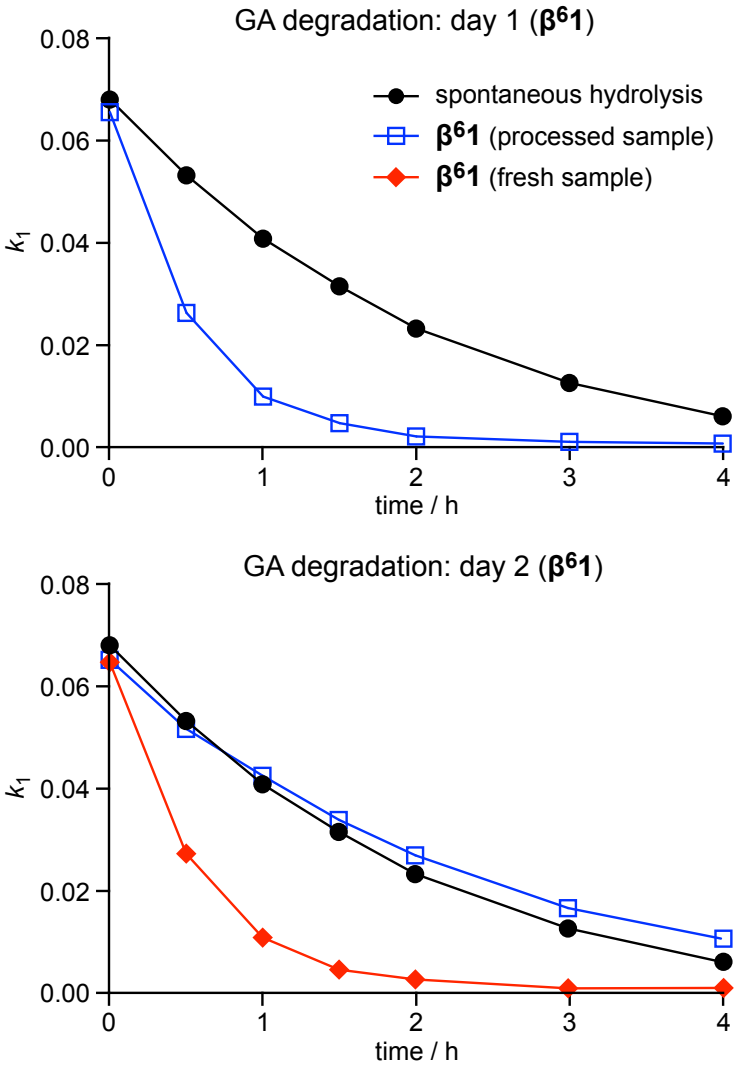


Figure S18: Hydroxamic acid **β^{6}1**.

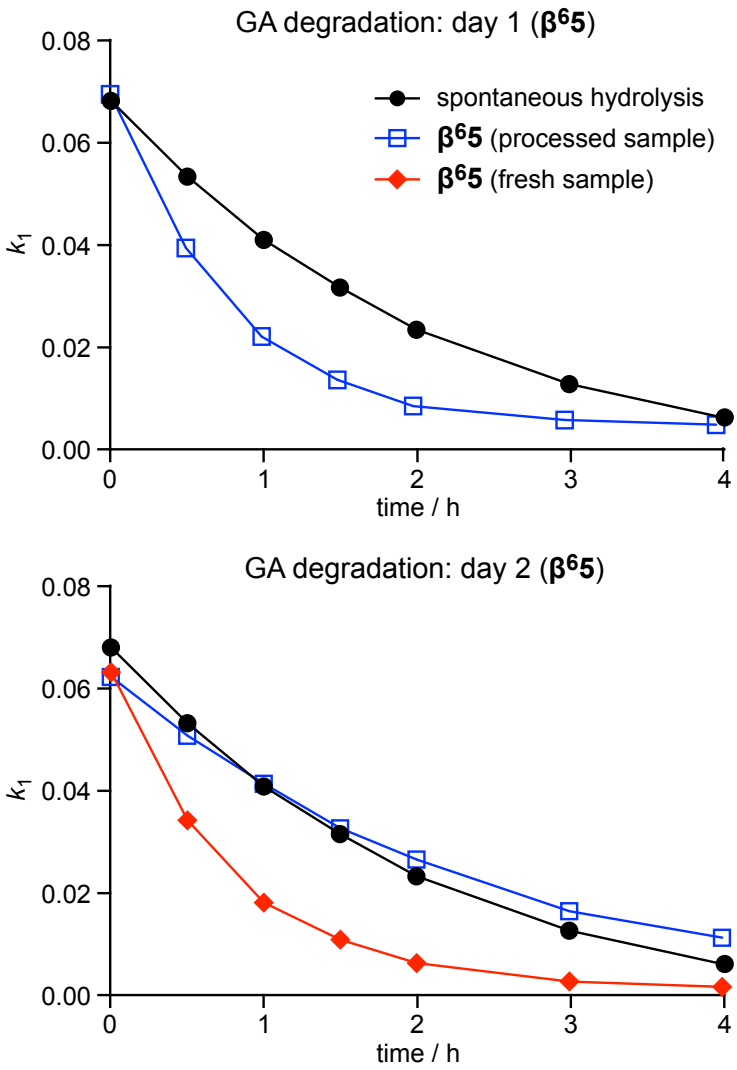


Figure S19: Hydroxamic acid **β^{6}5**.

4. GC-MS Assay

Quantitative GC-MS Assay. This assay[27] was performed in a stirred 2.0 mL cryo vial (Wheaton Science Products, Millville, NJ, USA) immersed in a temperature-controlled water bath (37.0 °C). GA (final concentration 1.1 µM) was added to buffer (1.85 mL, 0.1 M TRIS-HCl, pH 7.40, 37.0 °C) and an aliquot of the resulting solution (50 µL) was immediately removed to determine the initial concentration of GA enantiomers, $c_{(+)\text{-GA},0}$ and $c_{(-)\text{-GA},0}$. After adding a cyclodextrin solution pre-heated to 37.0 °C (200 µL, final concentration 500 µM) aliquots (50 µL) were taken at defined times and transferred into a tube containing ice-cold chloroform (1.50 mL). A solution of propyl dimethylphosphoramidocyanidate as internal standard (IS) in 2-propanol (100 ng/mL, 10 µL) was added immediately, the tube was shaken vigorously and placed on ice. Each sample was centrifuged (10,500 × g, 5 min, 4.0 °C) for 10 min, the organic layer removed, and transferred to a GC vial for analysis. The flowchart of this assay is depicted in Figure S20.

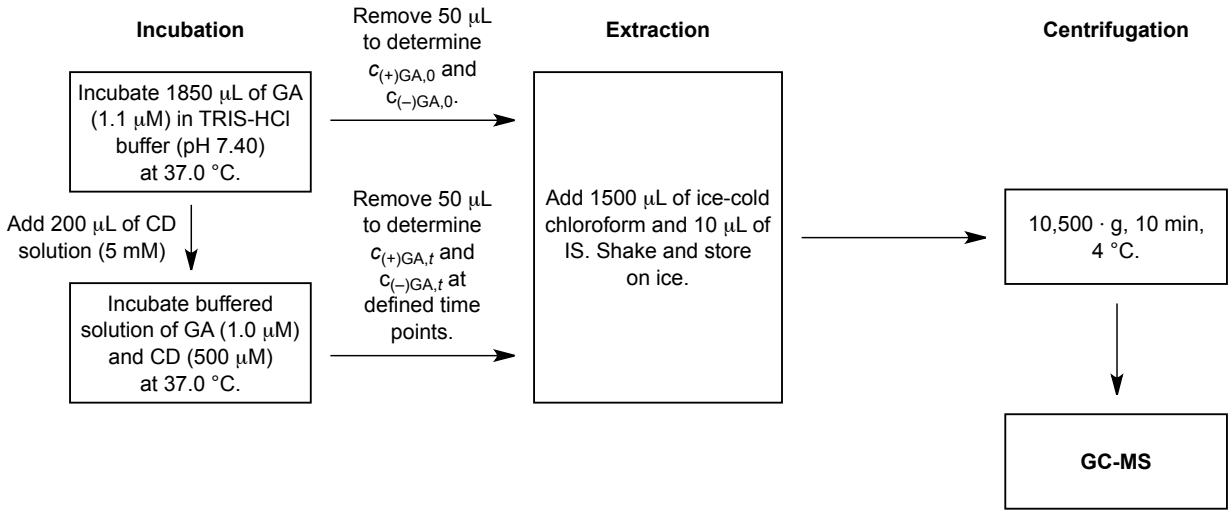


Figure S20: Flowchart showing the protocol of the quantitative GC-MS assay.

Quantification of GA enantiomers was performed by PCI-MS as described before[28] by using a 7890A gas chromatographic system coupled to a 5975C mass spectrometer detector with positive chemical ionisation (PCI) (Agilent Technologies, Waldbronn, Germany), large volume injection (LVI) and a CIS 4plus cold injection system (Gerstel, Mülheim an der Ruhr, Germany). Chromatographic separation was performed on a Supelco BetaDex™ 225 GC column (30 m × 0.25 mm, 0.25 µm film thickness, Sigma-Aldrich Chemie, Taufkirchen, Germany). Helium carrier gas was set at a constant flow of 1.3 mL/min. A solvent vent stop-flow injection mode was used. An aliquot of 40 µL was injected into an unpacked deactivated baffled siltek liner within 2.0 min at a

pre-column pressure of 0 bar (Table S1). The injector initial temperature was 50 °C. The final temperature of 200 °C was reached at a rate of 12 °C/s and was kept constant for 2.00 min. The initial time and vent time were set at 2.10 and 2.08 min, respectively. The vent flow rate was set at 10 mL/min, the purge flow rate at 50 mL/min with a purge time of 4.08 min. The column temperature program started at 50 °C which was held for 4.40 min. Then the temperature was raised to 170 °C with a rate of 12 °C/min and was maintained for 5.00 min. The following conditions were used for mass spectrometric analysis: positive chemical ionisation with ammonia 6.0 as reactant gas with a flow rate of 2.0 mL/min, transfer line temperature 230 °C, ion source temperature 210 °C, solvent delay 12.00 min. The target ions $[M+NH_4]^+$ were detected in the selected ion monitoring (SIM) mode (dwell time 125 ms each) at *m/z* 180 for the GA isomers and at *m/z* 194 for the IS isomers. The limit of quantification was estimated to be 5 pg/mL per enantiomer.

Table S1: GC-MS Parameters for PCI Analysis of GA.

Injection Program		MS parameters	
Injection volume	40 µL	Detected masses	*m/z* 180, *m/z* 194
Injection speed	20.0 µL/min	Dwell time	125 ms
Initial temperature	50.0 °C	Solvent delay	12.00 min
End temperature	200 °C, hold for 2.00 min		
Initial time	2.10 min		
Vent time	2.08 min		
Vent flow rate	10.0 mL/min		
Purge flow rate	50.0 mL/ min		
Purge time	4.08 min		

The experimental results were normalised to the initial concentration of GA in the absence of cyclodextrin, $c_{(+)\text{-GA},0}$ and $c_{(-)\text{-GA},0}$ by taking the difference in the initial GA concentration (1.1 µM versus 1.0 µM) into account. The kinetic constants k_{obs} were determined by non-linear regression analysis of the resulting decay curves (using GraphPad Prism 5.0, San Diego, CA, USA) on the basis of the following equation: $c_{(+)\text{-GA},t} = c_{(+)\text{-GA},0*} \cdot \exp(-k_{obs} \cdot t)$, in which $c_{(+)\text{-GA},t}$ denotes the concentration of (+)-GA at time *t* and $c_{(+)\text{-GA},0*}$ the concentration of (+)-GA immediately after cyclodextrin addition. Analogous equations were used for (–)-GA. Spontaneous rate of GA hydrolysis of was determined independently.

Figure S21 shows the time-dependent decrease of GA concentration (initial concentration 1 µM) mediated by $\beta^6\mathbf{1}_7$ (0.5 mM) at pH 7.40 and 37.0 °C monitored using the quantitative GC-MS assay (means of n = 2). The data points show the concentrations of (+)-GA and (–)-GA normalised to the

initial concentrations of both enantiomers, $c_{(+)\text{-GA},0}$ and $c_{(-)\text{-GA},0}$. (+)-GA is shown as blue squares and (–)-GA as red diamonds. The lines in show the curve fits of the measurements to a first-order rate reaction ($R^2 \geq 0.98$).

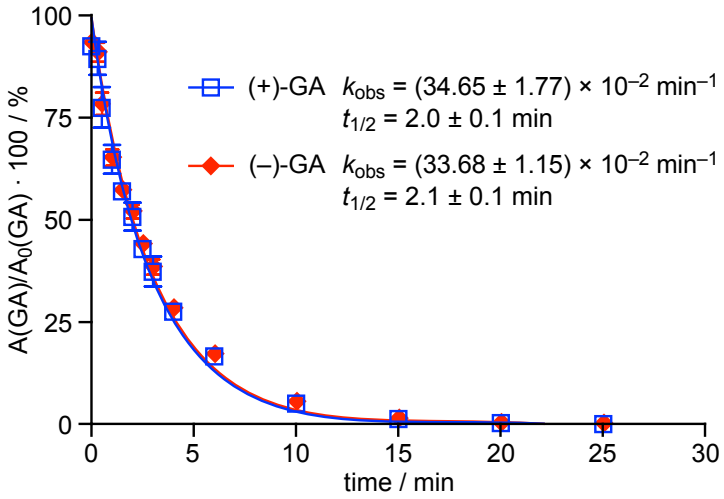


Figure S21: Result of the quantitative GC-MS assay.

5. NMR Assay

Spontaneous GA hydrolysis. GA (> 98% by ^{1}H NMR and ^{31}P NMR; 1 µL, final conc. 10.9 mM) was dissolved in an NMR tube in a mixture of D_2O (60 µL) and 0.1 M TRIS-HCl buffer (540 µL, pH 7.40). 48 Proton-decoupled ^{31}P NMR spectra were recorded from this sample at 310 K by using 512 scans per spectrum. 187 Dummy scans between each spectrum were used to set the interval between each recorded spectrum to 1801 s.

Three signals were visible in these spectra at 12.5, 11.4, and at –7.6 ppm (Figure S22). These signals were integrated within the ppm ranges –7.365 to –7.794 for **A**, 11.537 to 11.322 for **B**, 12.583 to 12.503 for **C**. The sum of the integrals for **B** and **C** at the end of the measurement were set to 100 %, and the relative ratios of the three compounds in each measurement were calculated accordingly. Figure S23 shows the development of the three signals with time.

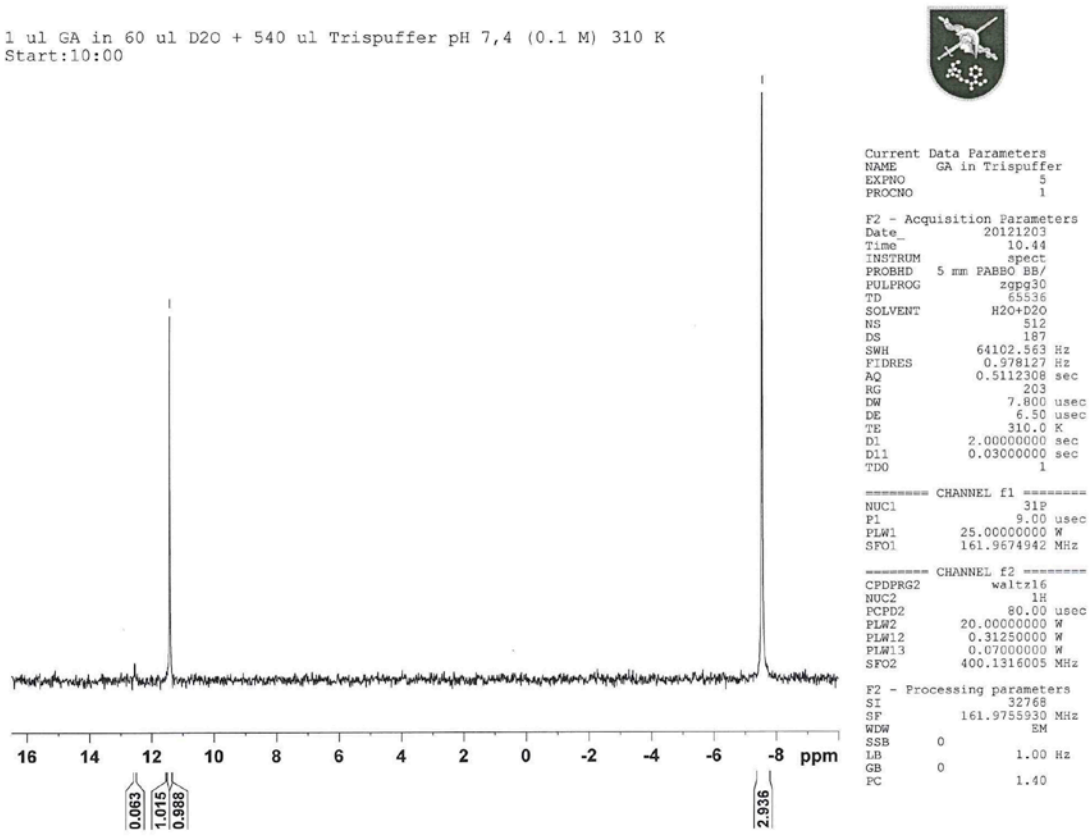


Figure S22: Representative ^{31}P NMR spectrum of GA (10.9 mM) after incubation in 0.1 M TRIS-HCl buffer (pH 7.40)/D_2O 9:1 (*v/v*) for 30 min at 37 °C.

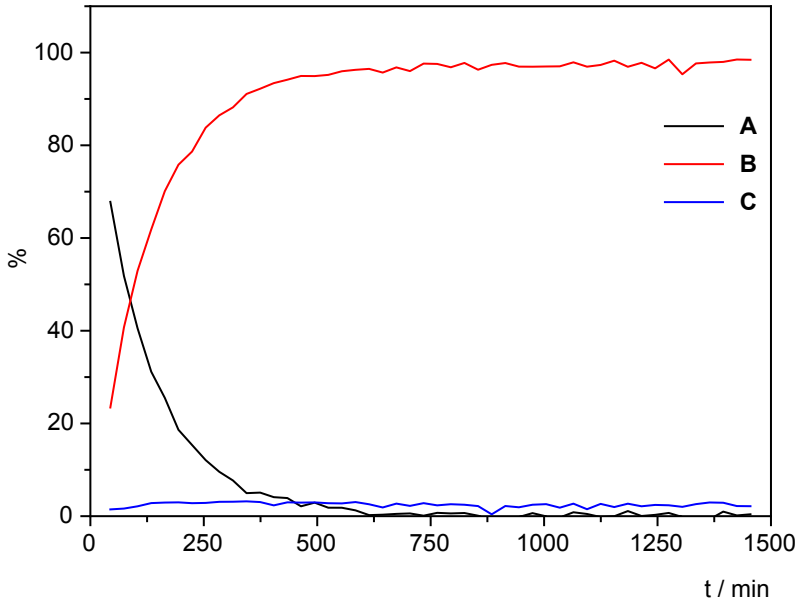
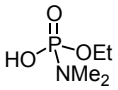


Figure S23: Spontaneous GA degradation during the NMR assay.

The signal at –7.6 ppm represents the peak of GA (**A**), which disappears progressively due to hydrolytic decomposition. ESI mass spectrometric analysis of the incubation solution revealed that GA hydrolysis mainly proceeds via cleavage of the CN group under the chosen conditions (Figure S24). Thus, the major product formed is ethyl dimethylphosphoramidate **B** (Chart S1), as confirmed by the ^{31}P NMR spectrum of an authentic sample of this compound (Figure S25). Other GA metabolites detected in the mass spectrum derive from cleavage of the dimethylamino group of GA or from cleavage of the CN and the ethoxy group. The small peak observed at 12.5 ppm in the ^{31}P NMR spectrum in Figure S22 is tentatively assigned to one of these compounds. Mass spectrometry provided no evidence for the formation of adducts between TRIS and GA.

Chart S1



B

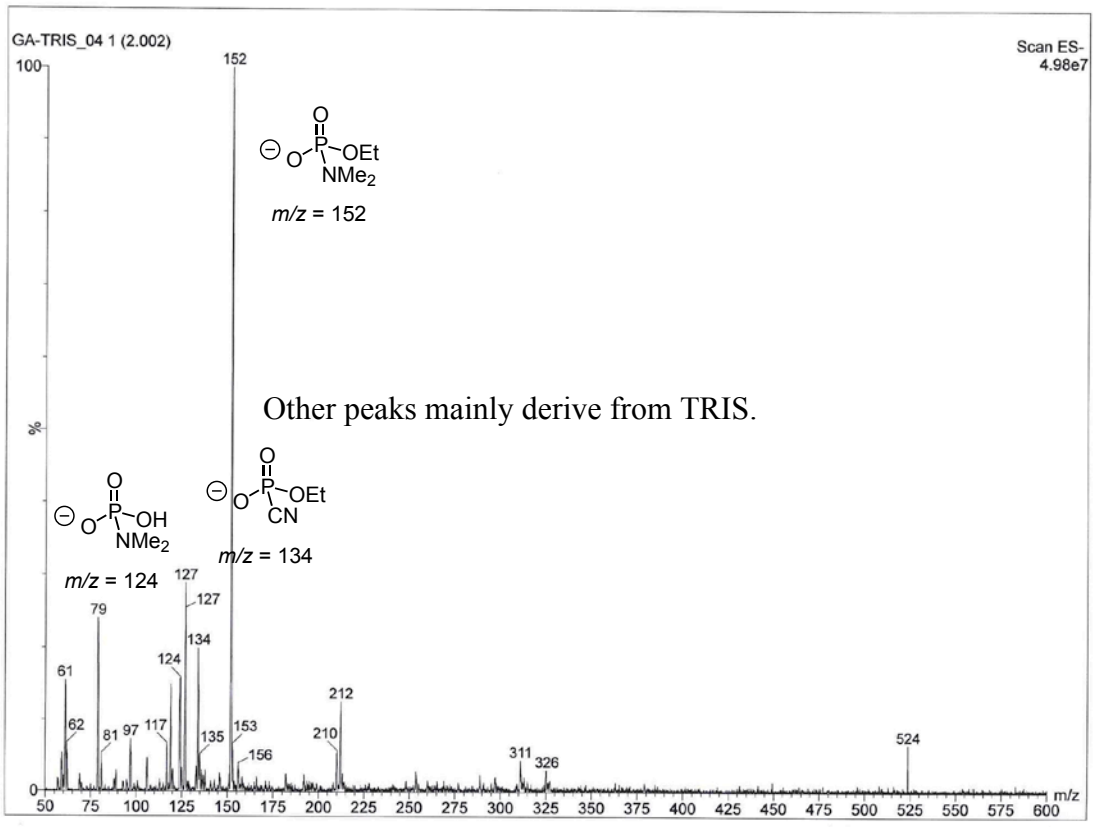


Figure S24: ESI-MS spectrum (negative mode) of the solution resulting after incubating GA in 0.1 M TRIS-HCl buffer (pH 7.40)/D_2O 9:1 (*v/v*) for 17.5 h at 37 °C.

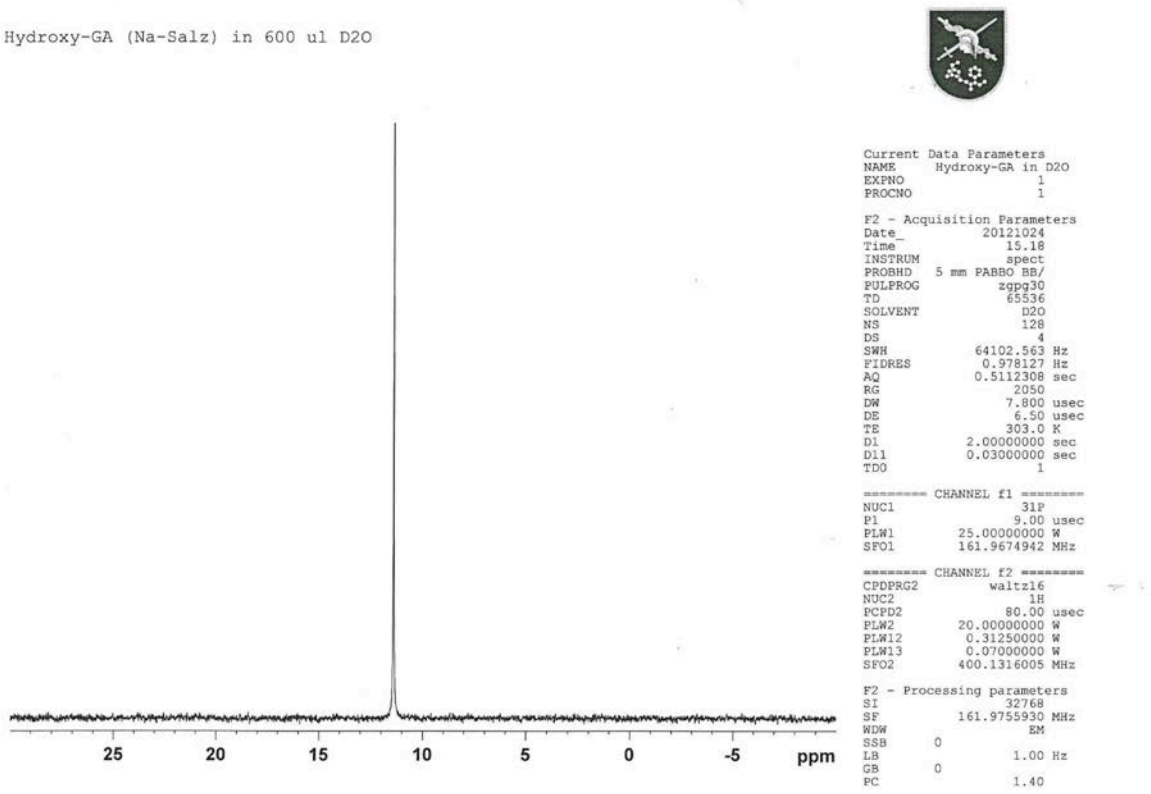


Figure S25: ^{31}P NMR spectrum of **B** in D_2O.

GA degradation in the presence of G^{6}1. G^{6}1 (4.0 mg, $C_{10}H_{16}N_4O_7$, M.W. = 304.26 g mol^{-1}, final conc. 21.9 mM) was dissolved in a mixture of D_2O (60 µL) and 0.1 M TRIS-HCl buffer (540 µL, pH 7.40). GA (> 98% by ^{1}H NMR and ^{31}P NMR; 1 µL, final conc. 10.9 mM) was added and the mixture shaken to ensure complete dissolution. Afterwards, the ^{31}P NMR spectra were recorded as described above.

The first NMR spectrum recorded after 33 min showed only two signals, one at 11.4 ppm and one at 2.3 ppm (Figure S26). The absence of a signal at –7.6 ppm is a clear indication that **G^{6}1** accelerates GA degradation beyond the rate of spontaneous hydrolysis. The signal at 11.4 ppm represents compound **B**, the major product of spontaneous GA degradation in TRIS-HCl buffer. In the presence of **G^{6}1**, **B** is only the minor species (2-3 %), however, formed presumably by spontaneous hydrolysis, whereas the signal at 2.3 ppm represents the major product. A mass spectrometric analysis of the incubation mixture (Figure S27) indicated that this product most likely represents compound **D** (Chart S2), resulting from condensation of **G^{6}1** and GA with loss of the CN and the NMe_2 group. The relative ratio of **B** and **D** did not change over the course of the

measurement. We assume that formation of **D** comprises initial phosphorylation of the hydroxamic acid OH group of $\mathbf{G^61}$ by GA, presumably involving loss of the CN group. The intermediate then hydrolyses by losing the NMe_2 group, a reaction that could be mediated intramolecularly by the neighboring hydroxamic acid group.

No peaks could be detected in the mass spectra indicating that reaction between GA and $\mathbf{G^61}$ would involve Lossen-rearrangement as observed when aryl hydroxamic acids react with organophosph(on)ates, in particular sarin.[29] $\mathbf{G^61}$ thus behaves like *N*-hydroxy carbamates, which also do not undergo Lossen-rearrangement after phosphorylation.[30]

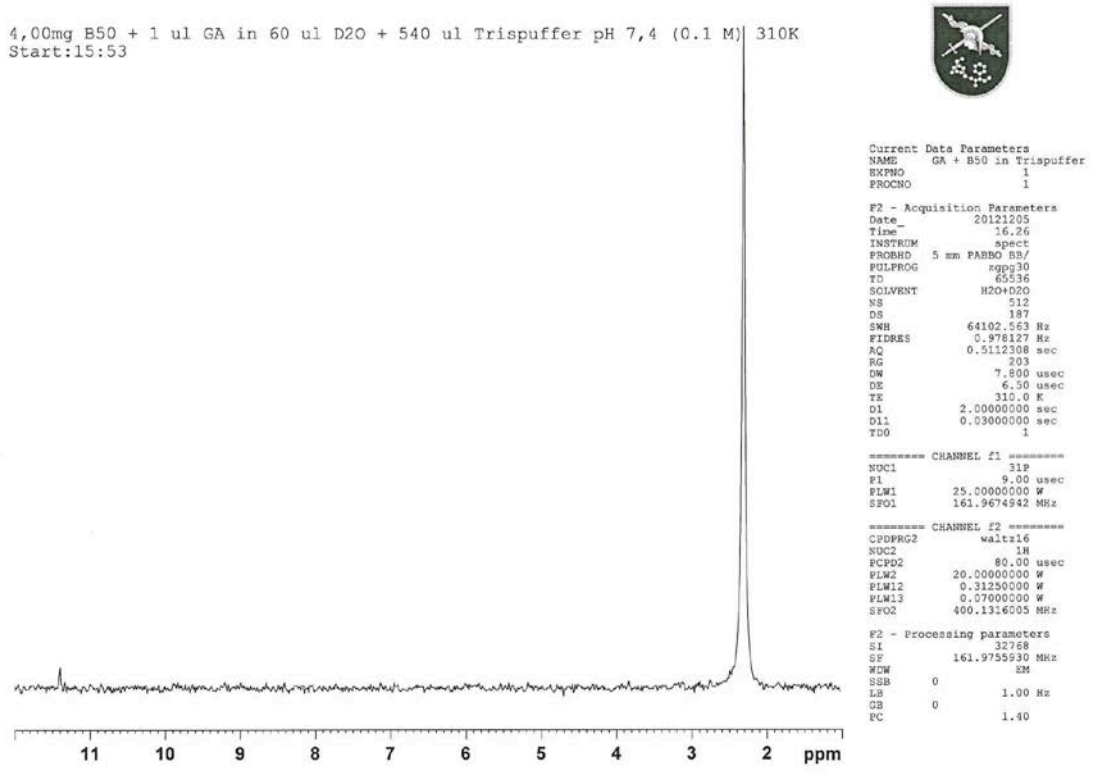
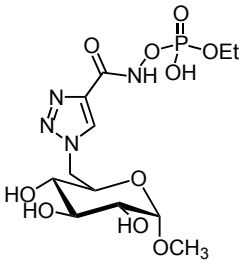


Figure S26: Representative ^{31}P NMR spectrum of GA (10.9 mM) after incubation in 0.1 M TRIS-HCl buffer (pH 7.40)/D_2O 9:1 (*v*/*v*) for 30 min at 37 °C in the presence of 2 equiv of $\mathbf{G^61}$.

Chart S2



D

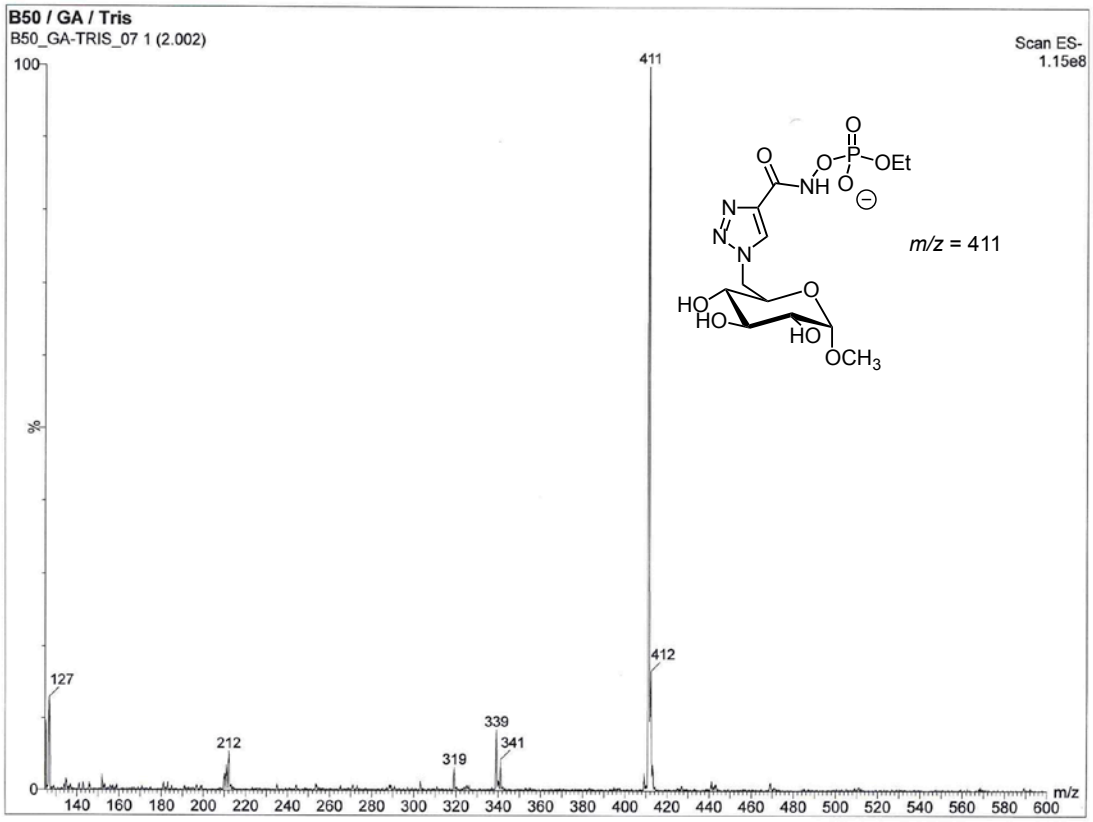


Figure S27: ESI-MS spectrum (negative mode) of the solution resulting after incubating GA with 2 equiv of **G^{6}1** in 0.1 M TRIS-HCl buffer (pH 7.40)/D_2O 9:1 (*v*/*v*) for 30 min at 37 °C.

GA degradation in the presence of G^{6}5. G^{6}5 (4.3 mg, $C_{11}H_{18}N_4O_7 \cdot 0.5\ H_2O$, M.W. = 327.79 g mol^{-1}, final conc. 21.9 mM) was dissolved in a mixture of D_2O (60 µL) and 0.1 M TRIS-HCl buffer (540 µL, pH 7.40). GA (> 98% by ^{1}H NMR and ^{31}P NMR; 1 µL, final conc. 10.9 mM) was added and the mixture shaken to ensure complete dissolution. Afterwards, the ^{31}P NMR spectra were recorded as described above.

Also in this case, there was no signal for GA visible even in the first spectrum recorded 33 min after preparing the sample, consistent with a substantial acceleration of GA degradation mediated by

G^{6}5. The mode of action of the *N*-methylated derivative **G^{6}5** turned out to be more complex than that of **G^{6}1**, however. In the first ^{31}P NMR spectrum three main signals are visible, two very close ones with resonances at 13.7 and 13.8 ppm (**E**) and one at 15.3 ppm (**F**) (Figure S28), in addition to a minor signal at 11.4 ppm representing compound **B**.

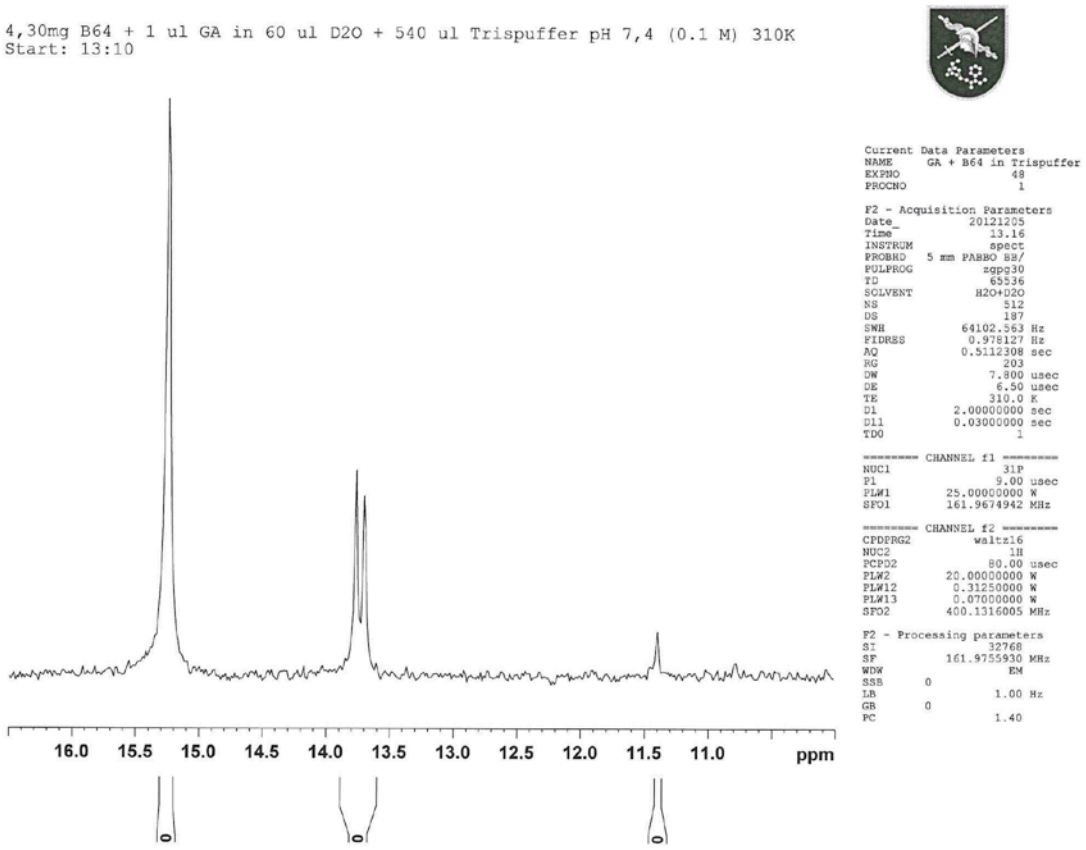


Figure S28: Representative ^{31}P NMR spectrum of GA (10.9 mM) after incubation in 0.1 M TRIS-HCl buffer (pH 7.40)/D_2O 9:1 (*v/v*) for 24 h at 37 °C in the presence of 2 equiv of **G^{6}5**.

The signals at 13.7 and 13.8 ppm became progressively smaller with time disappearing completely within 7 d. Compound **F** that gave rise to the signal at 15.3 ppm presumably only represents a transitional species as its signal grew during the first 2 d of the measurement and then slowly decreased again. A new signal appeared after 2 d at 0.7 ppm, which progressively increased and became the dominant signal in the final measurement (Figure S29) indicating that it could belong to the end product of the reaction (**G**). Additional signals appeared in the ^{31}P NMR spectra after prolonged reaction times at 10.8 and 2.9 ppm.

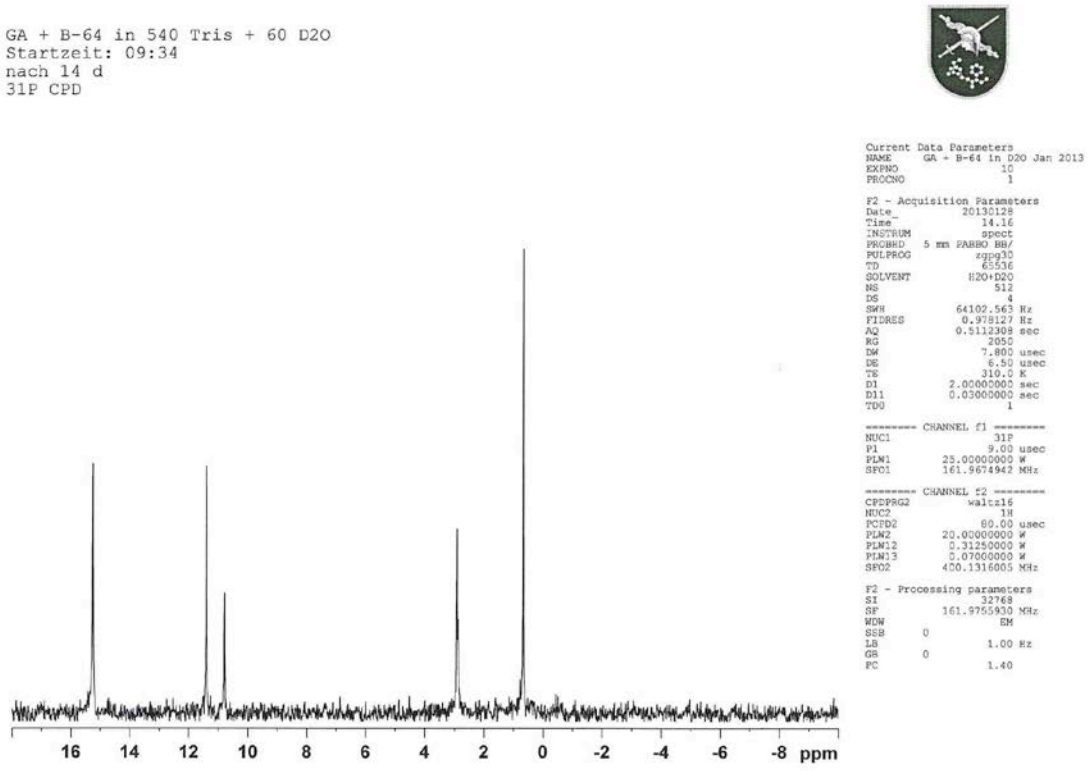
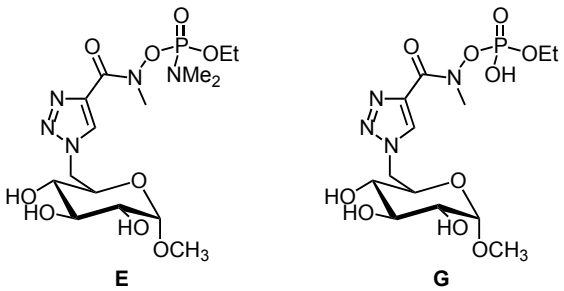


Figure S29: ^{31}P NMR spectrum of GA (10.9 mM) after incubation in 0.1 M TRIS-HCl buffer (pH 7.40)/D_2O 9:1 (*v/v*) for 14 d at 37 °C in the presence of 2 equiv of **G^{6}5**.

Mass spectrometric analysis of the incubation solution (Figure S30) provided strong evidence that the first step in the reaction between **G^{6}5** and GA affords phosphoramidate **E** (Chart S3). The fact that reaction between enantiopure **G^{6}5** and racemic GA leads to diastereomeric products explains why two signals with similar resonance frequencies were observed in the ^{31}P NMR for this compound.

Chart S3



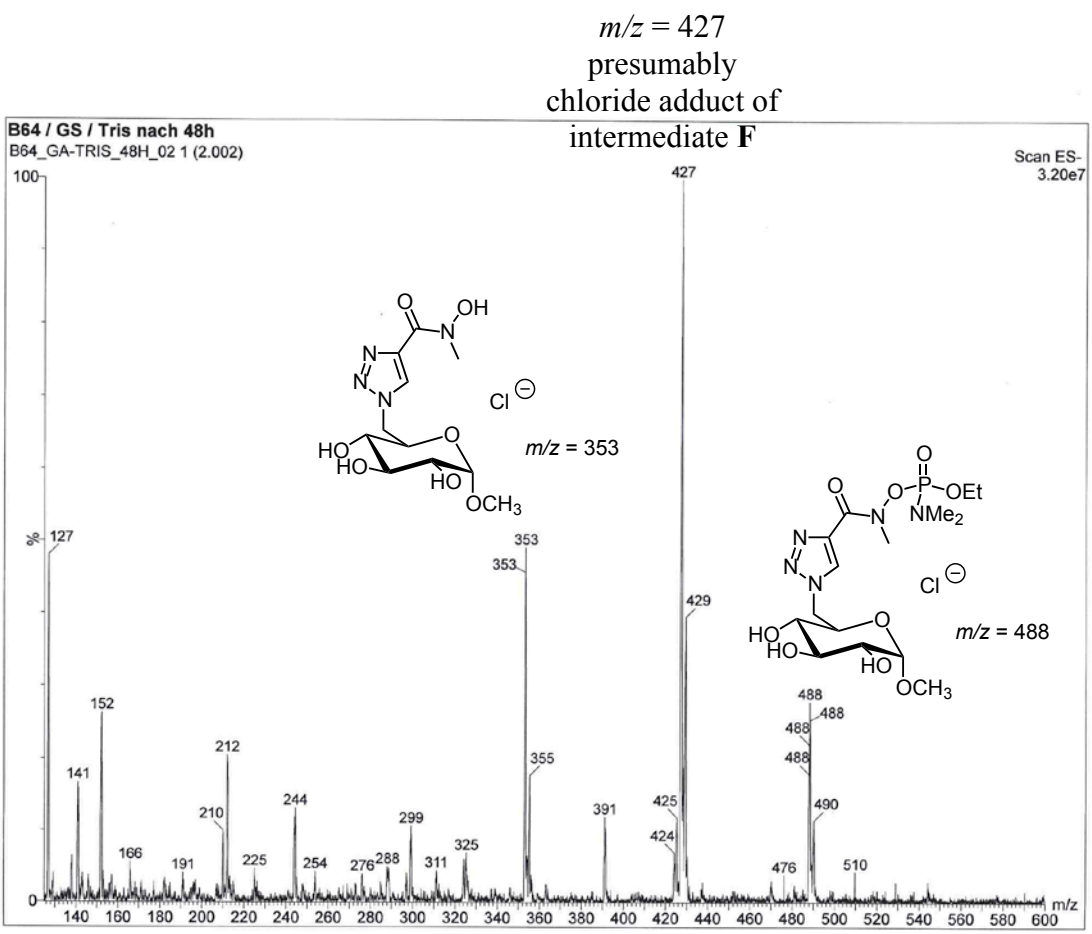


Figure S30: ESI-MS spectrum (negative mode) of the solution resulting after incubating GA with 2 equiv of **G**^{6}**5** in 0.1 M TRIS-HCl buffer (pH 7.40)/D_2O 9:1 (*v*/*v*) for 2 d at 37 °C.

In the mass spectra at the end of the reaction a prominent signal was observed at *m*/*z* = 425 (Figure S31). It therefore seems likely that the reaction eventually leads to **G**, which corresponds to the final product observed in the reaction between **G**^{6}**1** and GA. The complete reaction path leading to **G** including the nature of intermediate **F** has not been fully elucidated yet and is the object of ongoing investigations.

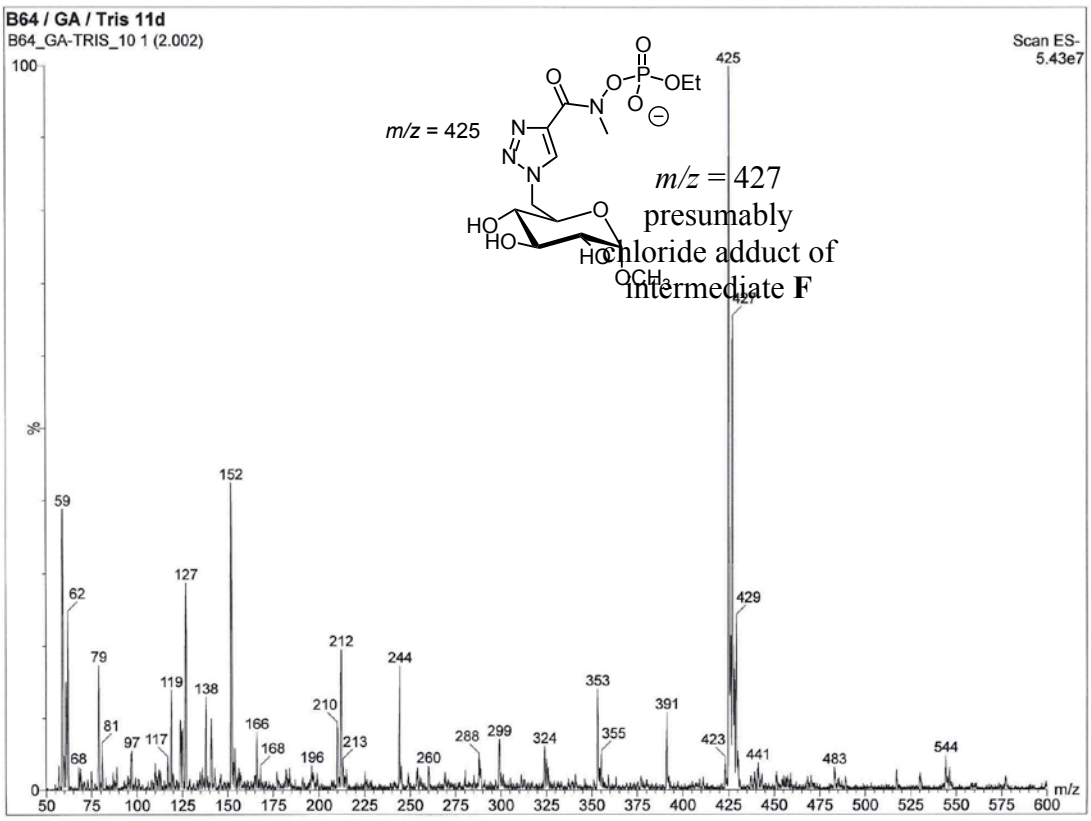


Figure S31: ESI-MS spectrum (negative mode) of the solution resulting after incubating GA with 2 equiv of **G^{6}5** in 0.1 M TRIS-HCl buffer (pH 7.40)/D_2O 9:1 (*v/v*) for 11 d at 37 °C.

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