# Examining the effects of vitamin $B_{12}$ conjugation on the biological activity of insulin: A molecular dynamic and *in vivo* oral uptake investigation

**Susan Clardy-James,[a] Damian G. Allis,[a] Timothy J. Fairchild*[b] and Robert P. Doyle*[a]**

***Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX***
**DOI: 10.1039/b000000x**

## Supplementary Information

Abbreviations

| | |
|---|---|
| **1** | $B_{12}$-B1-Insulin conjugate |
| **2** | $B_{12}$-B29-Insulin conjugate |
| $B_{12}$ | vitamin $B_{12}$ |
| BOC | di-*tert*-butyl dicarbonate |
| $CH_3CN$ | acetonitrile |
| FMOC | fluorenylmethyloxycarbonyl |
| MALDI-ToF MS | matrix-assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry |
| MD | molecular Dynamics |
| Min | minute |
| RP-HPLC | reverse phase high performance liquid chromatography |
| rt | retention time |
| TEA | triethylamine |
| TFA | trifluoroacetic acid |

**CONTENT**



List of Figures

Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications


## Experimental

**Materials and methods.** Chemicals and solvents were purchased from Sigma–Aldrich or Fluka and were used without further purification. Powdered bovine insulin was purchased from Sigma–Aldrich. Dimethyl sulfoxide (DMSO) was dried over 4 Å molecular sieves (200–400 mesh, Sigma) under dry dinitrogen. Dialysis tubing (7000 Da cutoff) was purchased from Pierce. $H_2O$ was distilled and deionized to 18.2 mΩ using a Barnstead Nano Diamond ultra purification machine. HPLC purification was carried out on an Agilent 1100 system with an Eclipse XDB-$C_{18}$ column (5 µm, 9.4 X 250 mm); flow rate: 1 mL/min. HPLC gradient system: solvent A: $dH_2O$/0.1% TFA; solvent B: $CH_3CN$/0.1% TFA; 0-10 min 75/25% A/B to 47.5/52.5% A/B. Matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was performed on a Bruker Autoflex III Smartbeam machine with laser intensity ranging from 50–73%. The matrix used was 10 mg α-cyano-4-hydroxycinnamic acid (CHCA) dissolved in $dH_2O/CH_3CN$ (1:1 v/v) containing 0.1% TFA. Such spectra were run with and without the presence of 10 mM dithiothreitol (DTT). Angiotensin I was used as internal control (m/z: 1296). Electron absorption spectra (EAS) were used to calculate concentrations and obtained on a Varian Cary 50 Bio spectrometer in a 1-mL quartz cuvette (Sigma) between 200 nm and 800 nm. All centrifugation was done at 4000 rpm for 10 minutes using a Sorvall centrifuge with swinging rotor (Sorvall Heraeus 75006441 K).

## Synthesis and Characterization

**Activated vitamin $B_{12}$.** The activation of the 5'-hydroxyl group of vitamin $B_{12}$ ($B_{12}$) was achieved using a previous reported method.[1] $B_{12}$ (10 mg, 0.007 mmol) and 1,1'-carbonyl-di-(1,2,4-triazole) (1.7 mg, 0.010 mol) were stirred in DMSO (1 mL, dried) under dry dinitrogen for 1 h. The activated $B_{12}$ was used without further purification.

**$B_{12}$-B1-Insulin (1)**. A variation of the literature procedure reported by Valliant *et al*. was used for the BOC protection of insulin.[2] BOC (4.0 mg, 0.0187 mmol) and *N*-hydroxysuccinimide (4.4 mg, 0.04 mmol) dissolved in DMSO (100 µL) containing 0.05% TEA (v/v) stirred for 0.5 h prior to being added to a stirring solution of bovine insulin (5.0 mg, 0.00087 mmol) dissolved in DMSO (1 mL) containing 0.05% TEA (v/v). After 0.5 h, an excess of activated $B_{12}$ (2.4 mg, 0.0017 mmol) was added to the protected insulin solution. The reaction stirred for 4 h and was isolated using diethyl ether ($Et_2O$) and centrifugation. The red solid was then dissolved in a 25/75 (v/v) $CH_3CN/H_2O$ containing 0.1% TFA and purified using analytical RP-HPLC. At a pressure of 71 bar, HPLC yielded a dark red fraction at rt = 1.4 min, pink fractions at 7.4 and 8.5 and clear fractions at rt=7.6, 8.1, 9.0 and 9.7. The fractions were then dissolved in TFA (0.5 ml) and stirred for 0.5 h to remove the BOC protection. The fractions were dialyzed against 5 L $dH_2O$ in snakeskin tubing (7000 Da cutoff). Dialysis against $H_2O$ removed residual free insulin and BOC. The dialysis bag content was then concentrated by lyophilization overnight. **1** (0.15 mg, 3%) was obtained as a light pink solid characterized by MALDI-ToF mass spectrometry. Concentration was calculated from EAS using $\varepsilon_{360}$ =27,291 $m^{-1}cm^{-1}$.

**$B_{12}$-B29-Insulin (2)**. Powder bovine insulin (5.0 mg, 0.00087 mmol) was dissolved in DMSO (1 mL) containing 0.05% TEA (v/v). An excess of activated $B_{12}$ (2.4 mg, 0.0017 mmol) was transferred to the unprotected insulin and reacted for 4 hours. FMOC-OSu (0.5 mg, 0.0015 mmol) in DMF (1 mL) was added to the reaction mixture. The reaction mixture stirred for 2 h and was isolated using $Et_2O$ and centrifugation. The red solid was then dissolved in a 25/75 (v/v) $CH_3CN/ H_2O$ containing 0.1% TFA and purified using analytical HPLC. At a pressure of 71 bar, HPLC yielded a dark red fraction at rt= 1.4 min, a pink fraction at 8.5 and clear fractions at rt=7.5 and 9.0 (Figure S1). Piperidine (10% v,v) was added to the pink fraction. Dialysis (7000 Da cut off) against $H_2O$ (5L) removed residual free insulin and FMOC. The dialysis bag content was then concentrated by lyophilization overnight. **2** (0.15 mg, 3%) was obtained as a light pink solid characterized by MALDI-ToF mass spectrometry. Concentration was calculated from EAS using $\varepsilon_{360}$ =27,291 $m^{-1}cm^{-1}$.

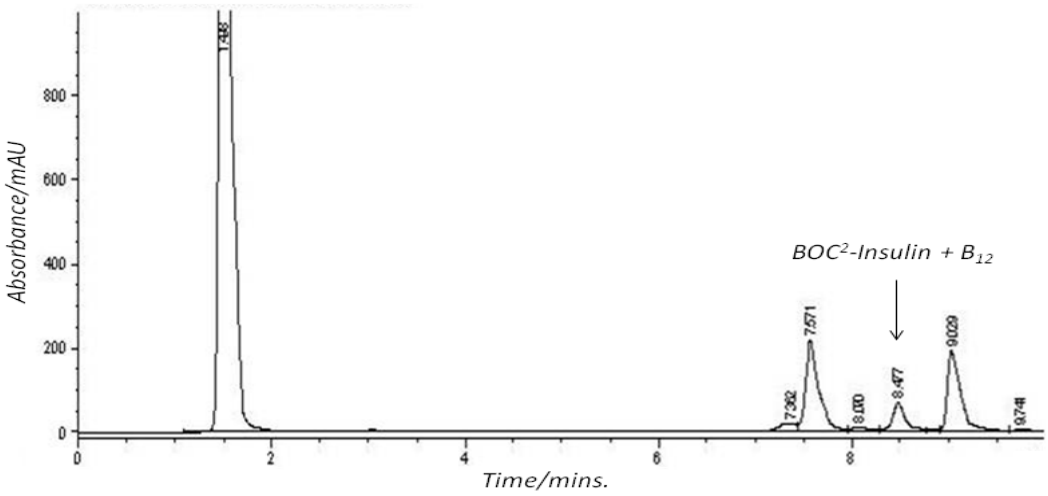


**Figure S1**. The RP-HPLC spectra of **2** monitored at 254 nm.

**MALDI-TOF of reduced and digested 1 and 2.** To reduce the disulfide bonds of insulin, the sample (0.050 mL) in water was diluted with aqueous $NH_4HCO_3$ (0.045 mL, 50 mM). To the diluted sample, dithiothreitol (5 µl, 100 mM) was added and incubated at 37°C for 1 hr. Digestion of the B chain was accomplished by the addition of a trypsin solution (10 µl, 1 mg/ml) to a reduced sample and incubation at 37°C overnight. Analysis of the reduced and trypsin digested samples were accomplished by MALDI-Tof analysis. The presence of 3844 m/z indicates $B_{12}$ attachment to the B1 position, while 2286 m/z or 2215 m/z indicate attachment at the B29 position.

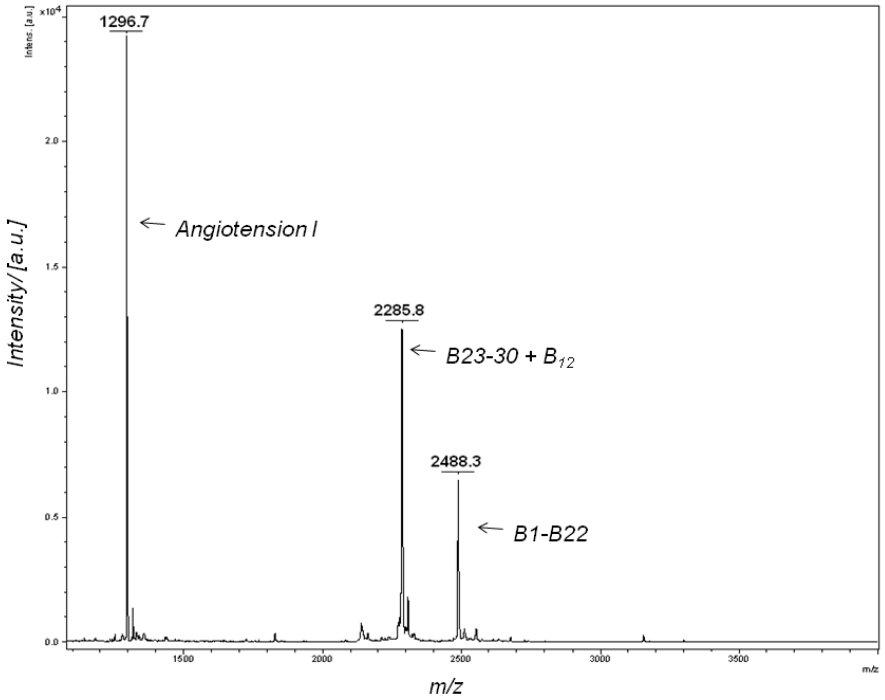


**Figure S2.** MALDI-Tof mass spectrometry spectrum of DTT reduced and tryptically digested **2** shows mass at 2286 m/z representing $B_{12}$ attached to B29 insulin.

**In vivo studies.** Male Sprague Dawley rats (279 ± 31; ± SD; n=12) were rendered insulin-deficient via subcutaneous injection of streptozotocin (STZ; 80 mg/kg BW) dissolved in citrate buffer (100mM; pH4.2). Blood glucose concentration was assessed after four days to ensure fasting levels of greater than 15 mmol/L. Maintenance of animals and experimental protocols were conducted in accordance with federal regulations and approved by the Murdoch University Animal Ethics Committee. Animals were fasted overnight prior to the first blood sample being taken from the lateral tail vein and assessed for glucose concentration (One-touch Verio). Compounds were then administered by oral gavage using a flexible feeding tube and blood samples collected 60, 120, 150, 180, 210 and 240min post gavage from the lateral tail vein. Area under the curve (AUC) transformations were conducted using the trapezoidal method in SigmaPlot, which was then subtracted from *pre-gavage[mmol] x 240min*; higher numbers represent a greater reduction in glucose concentration following oral gavage. Statistical significance was then assessed on this data using a one-way ANOVA with repeated measures (condition) after testing for normality (Shapiro-Wilk) and equal variance. Tukey *post Hoc* testing was used to assess differences between conditions.

**Molecular dynamics (MD) simulations.** A molecular dynamics (MD) simulation was performed using the GROMACS (v. 4.0.7)[3] software package with the GROMOS96[4](53a6) united-atom force field as the basis for the TCII and **2** parameters. The simulation was performed as an NPT ensemble (X,Y,Z) under periodic boundary conditions. The simulation temperature was kept constant at 300 K using a Berendsen thermostat[5] with 0.1 ps coupling time constants for solvent (flexible Simple Point Charge (SPC) water model + neutralizing counter-ions) and solute separately. The simulation pressure was kept constant using isotropic Berendsen barostats of 1.0 bar with a time coupling constant of 0.5 ps.[5] Electrostatic interactions were evaluated by the particle mesh Ewald (PME) method order 6 with a grid spacing of 0.1 nm in the X,Y,Z-directions.[6] The real-space and neighbor-search cut-off were set to 1.2 nm, with non-bonded pair lists updated every 10 steps. The MD simulation of **1**-TCII was performed with 1.0 fs timesteps for 15 ns in a 9.01840 nm cubic solvent box containing 20508 water molecules and 12 neutralizing $Na^+$ charges. Parameter generation, topology definitions, and structural data for $B_{12}$ and the **1** bridge used in the tether parameterization are an amalgam of quantum chemical, crystallographic, and pre-existing G53a6 force field data as defined previously.[1,7] The base structure and heavy atom labeling scheme for the $B_{12}$ topology definition are taken from the human $B_{12}$/TCII crystal structure (PDB 2BB5).[8] The coordination geometry of Co-CN and the Co-$N_{corrin}$ distances were adjusted based on the optimized CN-$B_{12}$ anion (ground-state singlet) structure at a B3LYP[9] level of theory with the 6-31G(d) basis set[10] using the program Gaussian03.[11] Force constants for all bond, angle, and torsion definitions for the Co atom and charge groups for the $B_{12}$ molecule are based on those in the G53a6 definition for Fe in the heme Fe-porphyrin ring. The combination of Co-N interatomic distances and Fe-N force constants is performed in the interest of computational expediency, with the rationale being that the difference in the stretch and bend terms at the center of the corrin ring are insignificant in the series of simulations, where $B_{12}$ is simply tethered in solution to the insulin B chain or bound within TCII and numerous hydrogen-bonding interactions between functional groups and the protein binding pocket dominate the interactions. This approach is extended to $B_{12}$ and **1**, where any possible TCII HIS-172/173 coordination to the open cobalt site is made impossible by the presence of $[CN]^-$. Angle and torsion definitions assure the proper orientation of aliphatic carbons in the corrin and ribose rings within the united-atom approximation. All remaining (non-$sp^3$ carbon) hydrogen atoms are defined explicitly in the structure topology. The complete set of GROMOS96 topologies for **1** and description of charge groups for this molecule for the force field are provided below. All images were rendered with VMD.[12]

Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications


# GROMOS96 Force Field Topology for B1-linked $B_{12}$-Insulin

Format below is GROMACS ffG53a6.rtp file-ready.

```
PCB - phe-cyanocobalamin residue file based on the HEMC CO parameters

[ PCB ]
[ atoms ]
 C27     C       0.29000     0    ;  from GLN residue
 O28     O      -0.45000     0    ;  from GLN residue
 N29     NT     -0.72000     0    ;  from GLN residue
 HAE     H       0.44000     0    ;  from GLN residue
 HAD     H       0.44000     0    ;  from GLN residue
 C32     C       0.29000     1    ;  from GLN residue
 O34     O      -0.45000     1    ;  from GLN residue
 N33     NT     -0.72000     1    ;  from GLN residue
 HAG     H       0.44000     1    ;  from GLN residue
 HAF     H       0.44000     1    ;  from GLN residue
 C38     C       0.29000     2    ;  from GLN residue
 O39     O      -0.45000     2    ;  from GLN residue
 N40     NT     -0.72000     2    ;  from GLN residue
 HAI     H       0.44000     2    ;  from GLN residue
 HAH     H       0.44000     2    ;  from GLN residue
 C43     C       0.29000     3    ;  from GLN residue
 O44     O      -0.45000     3    ;  from GLN residue
 N45     NT     -0.72000     3    ;  from GLN residue
 HAK     H       0.44000     3    ;  from GLN residue
 HAJ     H       0.44000     3    ;  from GLN residue
 C50     C       0.29000     4    ;  from GLN residue
 O51     O      -0.45000     4    ;  from GLN residue
 N52     NT     -0.72000     4    ;  from GLN residue
 HAM     H       0.44000     4    ;  from GLN residue
 HAL     H       0.44000     4    ;  from GLN residue
 C61     C       0.29000     5    ;  from GLN residue
 O63     O      -0.45000     5    ;  from GLN residue
 N62     NT     -0.72000     5    ;  from GLN residue
 HAO     H       0.44000     5    ;  from GLN residue
 HAN     H       0.44000     5    ;  from GLN residue
 C20     CH3     0.00000     6    ;  methyl group
 C25     CH3     0.00000     7    ;  methyl group
 C36     CH3     0.00000     8    ;  methyl group
 C46     CH3     0.00000     9    ;  methyl group
 C47     CH3     0.00000     10   ;  methyl group
 C54     CH3     0.00000     11   ;  methyl group
 C26     CH2     0.00000     12   ;  non-polar CHn fragments
 C30     CH2     0.00000     13   ;  non-polar CHn fragments
 C31     CH2     0.00000     13   ;  non-polar CHn fragments
 C37     CH2     0.00000     14   ;  non-polar CHn fragments
 C41     CH2     0.00000     15   ;  non-polar CHn fragments
 C42     CH2     0.00000     15   ;  non-polar CHn fragments
 C48     CH2     0.00000     16   ;  non-polar CHn fragments
 C49     CH2     0.00000     16   ;  non-polar CHn fragments
 C56     CH2     0.00000     17   ;  non-polar CHn fragments
 C55     CH2     0.00000     17   ;  non-polar CHn fragments
 C60     CH2     0.00000     18   ;  non-polar CHn fragments
 C2R     CH1     0.15000     19   ;  C2*-O2*-H2* from ADE
 O7R     OA     -0.54800     19   ;  C2*-O2*-H2* from ADE
 HAA     H       0.39800     19   ;  C2*-O2*-H2* from ADE
 C1R     CH1     0.20000     20   ;  C1*-O4*-C4* from ADE
 O6R     OA     -0.36000     20   ;  C1*-O4*-C4* from ADE
 C4R     CH1     0.16000     20   ;  C1*-O4*-C4* from ADE
 C3R     CH1     0.00000     21   ;  C3* from ADE
 C5R     CH2     0.15000     22   ;  C2*-O2*-H2* from ADE
 O8R     OA     -0.15000     22   ;  C2*-O2*-H2* from ADE  ;  to make the charge neutral
   N     N      -0.11000     22   ;  standard PHE (phe) atom information
   H     H       0.11000     22   ;  standard PHE (phe) atom information
   P     P       1.00000     23   ;  PO3+O3* fragment from ADE
 O2P     OA     -0.36500     23   ;  PO3+O3* fragment from ADE
 O4      OM     -0.63500     23   ;  PO3+O3* fragment from ADE
 O5      OM     -0.63500     23   ;  PO3+O3* fragment from ADE
 O3      OA     -0.36500     23   ;  PO3+O3* fragment from ADE
 C2P     CH1     0.00000     24   ;  non-polar CHn fragment
```

```
 C3P     CH3     0.00000     24   ;  non-polar CHn fragment
 C1P     CH2     0.00000     24   ;  non-polar CHn fragment
 N59     N      -0.30000     25   ;  tempered GLN fragment charges
 HAC     H       0.44000     25   ;  tempered GLN fragment charges
 C57     C       0.19000     25   ;  tempered GLN fragment charges
 O58     O      -0.33000     25   ;  tempered GLN fragment charges
 C5B     C       0.00000     26   ;  dimethylbenzimidazole C-CH3 group (non-polar)
 C5M     CH3     0.00000     26   ;  dimethylbenzimidazole C-CH3 group (non-polar)
 C6B     C       0.00000     27   ;  dimethylbenzimidazole C-CH3 group (non-polar)
 C6M     CH3     0.00000     27   ;  dimethylbenzimidazole C-CH3 group (non-polar)
 C4B     C      -0.14000     28   ;  dimethylbenzimidazole aromatic C-H from TRP, PHE
 H4B     H       0.14000     28   ;  dimethylbenzimidazole aromatic C-H from TRP, PHE
 C7B     C      -0.14000     29   ;  dimethylbenzimidazole aromatic C-H from TRP, PHE
 H7B     H       0.14000     29   ;  dimethylbenzimidazole aromatic C-H from TRP, PHE
 N3B     NR     -0.20000     30   ;  dimethylbenzimidazole polar aromatic NC fragment
 C9B     C       0.20000     30   ;  dimethylbenzimidazole polar aromatic NC fragment
 N1B     NR     -0.20000     31   ;  dimethylbenzimidazole polar aromatic NC fragment
 C8B     C       0.20000     31   ;  dimethylbenzimidazole polar aromatic NC fragment
 C2B     C       0.14000     32   ;  dimethylbenzimidazole aromatic C-H from TRP, PHE
 H2B     H      -0.14000     32   ;  dimethylbenzimidazole aromatic C-H from TRP, PHE
 C5      C       0.00000     33   ;  C-CH3 group (non-polar)
 C35     CH3     0.00000     33   ;  C-CH3 group (non-polar)
 C15     C       0.00000     34   ;  C-CH3 group (non-polar)
 C53     CH3     0.00000     34   ;  C-CH3 group (non-polar)
 FE      FE      0.60000     35   ;  HEMC Fe-N group
 N21     NR     -0.20000     35   ;  HEMC Fe-N group
 N22     NR     -0.10000     35   ;  HEMC Fe-N group
 N23     NR     -0.10000     35   ;  HEMC Fe-N group
 N24     NR     -0.20000     35   ;  HEMC Fe-N group
 C1      CH0     0.00000     36   ;  non-polar HEMC-derived corrin atoms
 C2      CH0     0.00000     36   ;  non-polar HEMC-derived corrin atoms
 C3      CH1     0.00000     36   ;  non-polar HEMC-derived corrin atoms
 C4      C       0.00000     36   ;  non-polar HEMC-derived corrin atoms
 C6      C       0.00000     37   ;  non-polar HEMC-derived corrin atoms
 C7      CH0     0.00000     37   ;  non-polar HEMC-derived corrin atoms
 C8      CH1     0.00000     37   ;  non-polar HEMC-derived corrin atoms
 C9      C       0.00000     37   ;  non-polar HEMC-derived corrin atoms
 C16     C       0.00000     38   ;  non-polar HEMC-derived corrin atoms
 C17     CH0     0.00000     38   ;  non-polar HEMC-derived corrin atoms
 C18     CH1     0.00000     38   ;  non-polar HEMC-derived corrin atoms
 C19     CH1     0.00000     38   ;  non-polar HEMC-derived corrin atoms
 C11     C       0.00000     39   ;  non-polar HEMC-derived corrin atoms
 C12     CH0     0.00000     39   ;  non-polar HEMC-derived corrin atoms
 C13     CH1     0.00000     39   ;  non-polar HEMC-derived corrin atoms
 C14     C       0.00000     39   ;  non-polar HEMC-derived corrin atoms
 C10     C      -0.14000     40   ;  aromatic C-H from TRP, PHE
 H10     H       0.14000     40   ;  aromatic C-H from TRP, PHE
  CA   CH1       0.00000     41   ;  standard PHE (phe) atom information
  CB   CH2       0.00000     41   ;  standard PHE (phe) atom information
  CG     C       0.00000     41   ;  standard PHE (phe) atom information
 CD1     C      -0.14000     42   ;  standard PHE (phe) atom information
 HD1    HC       0.14000     42   ;  standard PHE (phe) atom information
 CD2     C      -0.14000     43   ;  standard PHE (phe) atom information
 HD2    HC       0.14000     43   ;  standard PHE (phe) atom information
 CE1     C      -0.14000     44   ;  standard PHE (phe) atom information
 HE1    HC       0.14000     44   ;  standard PHE (phe) atom information
 CE2     C      -0.14000     45   ;  standard PHE (phe) atom information
 HE2    HC       0.14000     45   ;  standard PHE (phe) atom information
  CZ     C      -0.14000     46   ;  standard PHE (phe) atom information
  HZ    HC       0.14000     46   ;  standard PHE (phe) atom information
   C     C       0.45000     47   ;  standard PHE (phe) atom information
   O     O      -0.45000     47   ;  standard PHE (phe) atom information
 C1N     C       0.00000     48   ;  CN group on Fe
 N1C     N       0.00000     48   ;  CN group on Fe

[ bonds ]
;  ai    aj    gromos type
   C26   C27    gb_27   ;  from GLN fragment 0
   C27   O28    gb_5    ;  from GLN
   C27   N29    gb_9    ;  from GLN
   N29   HAE    gb_2    ;  from GLN
   N29   HAD    gb_2    ;  from GLN
   C31   C32    gb_27   ;  from GLN fragment 1
```

```
C32   O34    gb_5    ;  from GLN
C32   N33    gb_9    ;  from GLN
N33   HAG    gb_2    ;  from GLN
N33   HAF    gb_2    ;  from GLN
C37   C38    gb_27   ;  from GLN fragment 2
C38   O39    gb_5    ;  from GLN
C38   N40    gb_9    ;  from GLN
N40   HAI    gb_2    ;  from GLN
N40   HAH    gb_2    ;  from GLN
C42   C43    gb_27   ;  from GLN fragment 3
C43   O44    gb_5    ;  from GLN
C43   N45    gb_9    ;  from GLN
N45   HAK    gb_2    ;  from GLN
N45   HAJ    gb_2    ;  from GLN
C49   C50    gb_27   ;  from GLN fragment 4
C50   O51    gb_5    ;  from GLN
C50   N52    gb_9    ;  from GLN
N52   HAM    gb_2    ;  from GLN
N52   HAL    gb_2    ;  from GLN
C60   C61    gb_27   ;  from GLN fragment 5
C61   O63    gb_5    ;  from GLN
C61   N62    gb_9    ;  from GLN
N62   HAO    gb_2    ;  from GLN
N62   HAN    gb_2    ;  from GLN
FE    N21    gb_35   ;  FE-N bond from HEMC
FE    N22    gb_35   ;  FE-N bond from HEMC
FE    N23    gb_35   ;  FE-N bond from HEMC
FE    N24    gb_35   ;  FE-N bond from HEMC
FE    N3B    gb_37   ;  FE-N3B bond from HEMC not in corrin ring
P     O2P    gb_28   ;  from ADE phosphate
P     O4     gb_24   ;  from ADE phosphate
P     O5     gb_24   ;  from ADE phosphate
P     O3     gb_28   ;  from ADE phosphate
N21   C1     gb_21   ;  corrin ring N-CHn bonds
N21   C4     gb_14   ;  corrin ring N-C bonds
N22   C6     gb_14   ;  corrin ring N-C bonds
N22   C9     gb_14   ;  corrin ring N-C bonds
N23   C11    gb_14   ;  corrin ring N-C bonds
N23   C14    gb_14   ;  corrin ring N-C bonds
N24   C16    gb_14   ;  corrin ring N-C bonds
N24   C19    gb_21   ;  corrin ring N-CHn bonds
C30   C31    gb_27   ;  CH2-CH2 bonds
C41   C42    gb_27   ;  CH2-CH2 bonds
C48   C49    gb_27   ;  CH2-CH2 bonds
C55   C56    gb_27   ;  CH2-CH2 bonds
C56   C57    gb_27   ;  CH2-CH2 bonds
N1B   C1R    gb_22   ;  sugar bonds from ADE
C1R   C2R    gb_26   ;  sugar bonds from ADE
C2R   O7R    gb_20   ;  sugar bonds from ADE
O7R   HAA    gb_1    ;  sugar bonds from ADE
C2R   C3R    gb_26   ;  sugar bonds from ADE
C3R   C4R    gb_26   ;  sugar bonds from ADE
C3R   O2P    gb_20   ;  sugar bonds from ADE
C4R   C5R    gb_26   ;  sugar bonds from ADE
C5R   O8R    gb_20   ;  sugar bonds from ADE
C4R   O6R    gb_20   ;  sugar bonds from ADE
O6R   C1R    gb_20   ;  sugar bonds from ADE
C1P   N59    gb_9    ;  amide corrin - phosphate linkage
C1P   C2P    gb_27   ;  amide corrin - phosphate linkage
C2P   C3P    gb_27   ;  amide corrin - phosphate linkage
C2P   O3     gb_20   ;  amide corrin - phosphate linkage
N59   HAC    gb_2    ;  amide corrin - phosphate linkage
N59   C57    gb_9    ;  amide corrin - phosphate linkage
C57   O58    gb_5    ;  amide corrin - phosphate linkage
N1B   C2B    gb_10   ;  dimethylbenzimidazole ring
C2B   N3B    gb_10   ;  dimethylbenzimidazole ring
C2B   H2B    gb_3    ;  dimethylbenzimidazole ring
N3B   C9B    gb_10   ;  dimethylbenzimidazole ring
C9B   C4B    gb_16   ;  dimethylbenzimidazole ring
C4B   C5B    gb_16   ;  dimethylbenzimidazole ring
C4B   H4B    gb_3    ;  dimethylbenzimidazole ring
C5B   C6B    gb_16   ;  dimethylbenzimidazole ring
C5B   C5M    gb_27   ;  dimethylbenzimidazole ring
```

```
   C6B   C7B    gb_16   ;  dimethylbenzimidazole ring
   C6B   C6M    gb_27   ;  dimethylbenzimidazole ring
   C7B   C8B    gb_16   ;  dimethylbenzimidazole ring
   C7B   H7B    gb_3    ;  dimethylbenzimidazole ring
   C8B   N1B    gb_10   ;  dimethylbenzimidazole ring
   C8B   C9B    gb_16   ;  dimethylbenzimidazole ring
   C1    C20    gb_27   ;  corrin ring carbon framework
   C1    C2     gb_27   ;  corrin ring carbon framework
   C2    C3     gb_27   ;  corrin ring carbon framework
   C2    C25    gb_27   ;  corrin ring carbon framework
   C2    C26    gb_27   ;  corrin ring carbon framework
   C3    C4     gb_27   ;  corrin ring carbon framework
   C3    C30    gb_27   ;  corrin ring carbon framework
   C4    C5     gb_17   ;  corrin ring carbon framework
   C5    C6     gb_17   ;  corrin ring carbon framework
   C5    C35    gb_27   ;  corrin ring carbon framework
   C6    C7     gb_27   ;  corrin ring carbon framework
   C7    C8     gb_27   ;  corrin ring carbon framework
   C7    C37    gb_27   ;  corrin ring carbon framework
   C7    C36    gb_27   ;  corrin ring carbon framework
   C8    C9     gb_27   ;  corrin ring carbon framework
   C8    C41    gb_27   ;  corrin ring carbon framework
   C9    C10    gb_17   ;  corrin ring carbon framework
   C10   C11    gb_17   ;  corrin ring carbon framework
   C11   C12    gb_27   ;  corrin ring carbon framework
   C12   C46    gb_27   ;  corrin ring carbon framework
   C12   C47    gb_27   ;  corrin ring carbon framework
   C12   C13    gb_27   ;  corrin ring carbon framework
   C13   C14    gb_27   ;  corrin ring carbon framework
   C13   C48    gb_27   ;  corrin ring carbon framework
   C14   C15    gb_17   ;  corrin ring carbon framework
   C15   C16    gb_17   ;  corrin ring carbon framework
   C15   C53    gb_27   ;  corrin ring carbon framework
   C16   C17    gb_27   ;  corrin ring carbon framework
   C17   C18    gb_27   ;  corrin ring carbon framework
   C17   C54    gb_27   ;  corrin ring carbon framework
   C17   C55    gb_27   ;  corrin ring carbon framework
   C18   C19    gb_27   ;  corrin ring carbon framework
   C18   C60    gb_27   ;  corrin ring carbon framework
   C19   C1     gb_27   ;  corrin ring carbon framework
   C10   H10    gb_3    ;  corrin ring C10-H10 bond
    N     H     gb_2    ;  standard PHE (phenylalanine)
    N    CA     gb_21   ;  standard PHE (phenylalanine)
   CA    CB     gb_27   ;  standard PHE (phenylalanine)
   CA     C     gb_27   ;  standard PHE (phenylalanine)
   CB    CG     gb_27   ;  standard PHE (phenylalanine)
   CG   CD1     gb_16   ;  standard PHE (phenylalanine)
   CG   CD2     gb_16   ;  standard PHE (phenylalanine)
  CD1   HD1     gb_3    ;  standard PHE (phenylalanine)
  CD1   CE1     gb_16   ;  standard PHE (phenylalanine)
  CD2   HD2     gb_3    ;  standard PHE (phenylalanine)
  CD2   CE2     gb_16   ;  standard PHE (phenylalanine)
  CE1   HE1     gb_3    ;  standard PHE (phenylalanine)
  CE1    CZ     gb_16   ;  standard PHE (phenylalanine)
  CE2   HE2     gb_3    ;  standard PHE (phenylalanine)
  CE2    CZ     gb_16   ;  standard PHE (phenylalanine)
   CZ    HZ     gb_3    ;  standard PHE (phenylalanine)
    C     O     gb_5    ;  standard PHE (phenylalanine)
    C    +N     gb_10   ;  standard PHE (phenylalanine)
   O8R   N    gb_5    ;  PHE B12 linker (CLB replaces H)
   FE   C1N    gb_30   ;  cyano group on the Fe
  C1N   N1C    gb_4    ;  cyano group on the Fe

    [ exclusions ]
;  ai    aj
   FE    C2
   FE    C3
   FE    C5
   FE    C7
   FE    C8
   FE    C10
   FE    C12
   FE    C13
```

```
 FE    C15
 FE    C17
 FE    C18
 FE    C20
 N21   C25
 N21   C26
 N21   C30
 N21   C35
 N21   C6
 N21   C9
 N21   C11
 N21   C14
 N21   C16
 N21   C19
 N21   C10
 N22   C1
 N22   C4
 N22   C36
 N22   C37
 N22   C11
 N22   C14
 N22   C16
 N22   C19
 N22   C15
 N23   C16
 N23   C19
 N23   C1
 N23   C4
 N23   C6
 N23   C9
 N23   C46
 N23   C47
 N23   C48
 N23   C5
 N24   C4
 N24   C5
 N24   C6
 N24   C9
 N24   C10
 N24   C11
 N24   C14
 N24   C55
 N24   C54
 N24   C60
 C1R   C7B
 C1R   C9B
 C1R   N3B
 N1B   C6B
 N1B   C4B
 C8B   C5B
 C8B   C5M
 C8B   C6M
 C7B   C4B
 C7B   N3B
 C7B   C2B
 C2B   C9B
 C5B   C2B
 C5M   C6M
 CB    HD1    ; PHE exclusions from standard topology setting
 CB    HD2    ; PHE exclusions from standard topology setting
 CB    CE1    ; PHE exclusions from standard topology setting
 CB    CE2    ; PHE exclusions from standard topology setting
 CG    HE1    ; PHE exclusions from standard topology setting
 CG    HE2    ; PHE exclusions from standard topology setting
 CG     CZ    ; PHE exclusions from standard topology setting
CD1    HD2    ; PHE exclusions from standard topology setting
CD1    CE2    ; PHE exclusions from standard topology setting
CD1     HZ    ; PHE exclusions from standard topology setting
HD1    CD2    ; PHE exclusions from standard topology setting
HD1    HE1    ; PHE exclusions from standard topology setting
HD1     CZ    ; PHE exclusions from standard topology setting
CD2    CE1    ; PHE exclusions from standard topology setting
CD2     HZ    ; PHE exclusions from standard topology setting
```

```
  HD2   HE2    ; PHE exclusions from standard topology setting
  HD2    CZ    ; PHE exclusions from standard topology setting
  CE1   HE2    ; PHE exclusions from standard topology setting
  HE1   CE2    ; PHE exclusions from standard topology setting
  HE1    HZ    ; PHE exclusions from standard topology setting
  HE2    HZ    ; PHE exclusions from standard topology setting
   C1    C30   ;  HEMC-based C framework exclusions
   C1    C5    ;  HEMC-based C framework exclusions
   C2    C5    ;  HEMC-based C framework exclusions
   C3    C35   ;  HEMC-based C framework exclusions
   C3    C6    ;  HEMC-based C framework exclusions
   C4    C26   ;  HEMC-based C framework exclusions
   C4    C7    ;  HEMC-based C framework exclusions
   C30   C26   ;  HEMC-based C framework exclusions
   C30   C5    ;  HEMC-based C framework exclusions
   C5    C8    ;  HEMC-based C framework exclusions
   C5    C9    ;  HEMC-based C framework exclusions
   C5    C36   ;  HEMC-based C framework exclusions
   C5    C37   ;  HEMC-based C framework exclusions
   C35   C7    ;  HEMC-based C framework exclusions
   C6    C41   ;  HEMC-based C framework exclusions
   C6    C10   ;  HEMC-based C framework exclusions
   C7    C10   ;  HEMC-based C framework exclusions
   C8    H10   ;  HEMC-based C framework exclusions
   C8    C11   ;  HEMC-based C framework exclusions
   C9    C36   ;  HEMC-based C framework exclusions
   C9    C37   ;  HEMC-based C framework exclusions
   C9    C12   ;  HEMC-based C framework exclusions
   C36   C41   ;  HEMC-based C framework exclusions
   C37   C10   ;  HEMC-based C framework exclusions
   C10   C13   ;  HEMC-based C framework exclusions
   C10   C14   ;  HEMC-based C framework exclusions
   C10   C46   ;  HEMC-based C framework exclusions
   C10   C47   ;  HEMC-based C framework exclusions
   H10   C12   ;  HEMC-based C framework exclusions
   C11   C48   ;  HEMC-based C framework exclusions
   C11   C15   ;  HEMC-based C framework exclusions
   C12   C15   ;  HEMC-based C framework exclusions
   C13   C53   ;  HEMC-based C framework exclusions
   C13   C16   ;  HEMC-based C framework exclusions
   C14   C46   ;  HEMC-based C framework exclusions
   C14   C47   ;  HEMC-based C framework exclusions
   C14   C17   ;  HEMC-based C framework exclusions
   C46   C48   ;  HEMC-based C framework exclusions
   C47   C48   ;  HEMC-based C framework exclusions
   C48   C15   ;  HEMC-based C framework exclusions
   C15   C18   ;  HEMC-based C framework exclusions
   C15   C19   ;  HEMC-based C framework exclusions
   C15   C54   ;  HEMC-based C framework exclusions
   C15   C55   ;  HEMC-based C framework exclusions
   C53   C17   ;  HEMC-based C framework exclusions
   C16   C60   ;  HEMC-based C framework exclusions
   C19   C54   ;  HEMC-based C framework exclusions
   C19   C55   ;  HEMC-based C framework exclusions
   C54   C60   ;  HEMC-based C framework exclusions
   C55   C60   ;  HEMC-based C framework exclusions
;
; cyano group addition to the original B12 topology
;
   N21   N1C   ;  cyano exclusions from HEMC CO group
   N22   N1C   ;  cyano exclusions from HEMC CO group
   N23   N1C   ;  cyano exclusions from HEMC CO group
   N24   N1C   ;  cyano exclusions from HEMC CO group
   C1    C1N   ;  cyano exclusions from HEMC CO group
   C4    C1N   ;  cyano exclusions from HEMC CO group
   C6    C1N   ;  cyano exclusions from HEMC CO group
   C9    C1N   ;  cyano exclusions from HEMC CO group
   C11   C1N   ;  cyano exclusions from HEMC CO group
   C14   C1N   ;  cyano exclusions from HEMC CO group
   C16   C1N   ;  cyano exclusions from HEMC CO group
   C19   C1N   ;  cyano exclusions from HEMC CO group
   N3B   N1C   ;  cyano exclusion unique to 6-coordinate iron
   C1N   C9B   ;  additional cyano exclusion at Fe 6-center
```

```
   C1N   C2B   ;  additional cyano exclusion at Fe 6-center

 [ angles ]
;  ai    aj    ak   gromos type
   C2    C26   C27   ga_15   ;  from GLN, fragment 1
   C26   C27   O28   ga_30   ;  from GLN
   C26   C27   N29   ga_19   ;  from GLN
   O28   C27   N29   ga_33   ;  from GLN
   C27   N29   HAE   ga_23   ;  from GLN
   C27   N29   HAD   ga_23   ;  from GLN
   HAE   N29   HAD   ga_24   ;  from GLN
   C30   C31   C32   ga_15   ;  from GLN, fragment 2
   C31   C32   O34   ga_30   ;  from GLN
   C31   C32   N33   ga_19   ;  from GLN
   O34   C32   N33   ga_33   ;  from GLN
   C32   N33   HAG   ga_23   ;  from GLN
   C32   N33   HAF   ga_23   ;  from GLN
   HAG   N33   HAF   ga_24   ;  from GLN
   C7    C37   C38   ga_15   ;  from GLN, fragment 3
   C37   C38   O39   ga_30   ;  from GLN
   C37   C38   N40   ga_19   ;  from GLN
   O39   C38   N40   ga_33   ;  from GLN
   C38   N40   HAI   ga_23   ;  from GLN
   C38   N40   HAH   ga_23   ;  from GLN
   HAI   N40   HAH   ga_24   ;  from GLN
   C41   C42   C43   ga_15   ;  from GLN, fragment 4
   C42   C43   O44   ga_30   ;  from GLN
   C42   C43   N45   ga_19   ;  from GLN
   O44   C43   N45   ga_33   ;  from GLN
   C43   N45   HAK   ga_23   ;  from GLN
   C43   N45   HAJ   ga_23   ;  from GLN
   HAK   N45   HAJ   ga_24   ;  from GLN
   C48   C49   C50   ga_15   ;  from GLN, fragment 5
   C49   C50   O51   ga_30   ;  from GLN
   C49   C50   N52   ga_19   ;  from GLN
   O51   C50   N52   ga_33   ;  from GLN
   C50   N52   HAM   ga_23   ;  from GLN
   C50   N52   HAL   ga_23   ;  from GLN
   HAM   N52   HAL   ga_24   ;  from GLN
   C18   C60   C61   ga_15   ;  from GLN, fragment 6
   C60   C61   O63   ga_30   ;  from GLN
   C60   C61   N62   ga_19   ;  from GLN
   O63   C61   N62   ga_33   ;  from GLN
   C61   N62   HAO   ga_23   ;  from GLN
   C61   N62   HAN   ga_23   ;  from GLN
   HAO   N62   HAN   ga_24   ;  from GLN
   N21   FE    N22   ga_2    ;  corrin ring Fe-N angle
   N21   FE    N24   ga_2    ;  corrin ring Fe-N angle
   N22   FE    N23   ga_2    ;  corrin ring Fe-N angle
   N23   FE    N24   ga_2    ;  corrin ring Fe-N angle
   N21   FE    N3B   ga_2    ;  corrin-Fe-dimethylbenzimidazole
   N22   FE    N3B   ga_2    ;  corrin-Fe-dimethylbenzimidazole
   N23   FE    N3B   ga_2    ;  corrin-Fe-dimethylbenzimidazole
   N24   FE    N3B   ga_2    ;  corrin-Fe-dimethylbenzimidazole
   FE    N21   C1    ga_34   ;  corrin Fe-N-C angles (based on HEMC)
   FE    N21   C4    ga_34   ;  corrin Fe-N-C angles (based on HEMC)
   FE    N22   C6    ga_34   ;  corrin Fe-N-C angles (based on HEMC)
   FE    N22   C9    ga_34   ;  corrin Fe-N-C angles (based on HEMC)
   FE    N23   C11   ga_34   ;  corrin Fe-N-C angles (based on HEMC)
   FE    N23   C14   ga_34   ;  corrin Fe-N-C angles (based on HEMC)
   FE    N24   C16   ga_34   ;  corrin Fe-N-C angles (based on HEMC)
   FE    N24   C19   ga_34   ;  corrin Fe-N-C angles (based on HEMC)
   FE    N3B   C9B   ga_34   ;  Fe-dimethylbenzimidazole-N,C angle
   FE    N3B   C2B   ga_34   ;  Fe-dimethylbenzimidazole-N,C angle
   C8B   N1B   C2B   ga_7    ;  angle containing C8B, see minimization issues discussion
   N1B   C8B   C9B   ga_7    ;  angle containing C8B, see minimization issues discussion
   C8B   C9B   N3B   ga_7    ;  angle containing C8B, see minimization issues discussion
   C7B   C8B   N1B   ga_39   ;  angle containing C8B, see minimization issues discussion
   C8B   N1B   C1R   ga_37   ;  angle containing C8B, see minimization issues discussion
   C4B   C9B   C8B   ga_27   ;  angle containing C8B, see minimization issues discussion
   C6B   C7B   C8B   ga_27   ;  angle containing C8B, see minimization issues discussion
   C7B   C8B   C9B   ga_27   ;  angle containing C8B, see minimization issues discussion
   N21   C1    C2    ga_13   ;  corrin ring-specific but HEMC-derived angles
```

```
N21   C1    C20   ga_13   ;  corrin ring-specific but HEMC-derived angles
N21   C4    C3    ga_33   ;  corrin ring-specific but HEMC-derived angles
C4    N21   C1    ga_6    ;  corrin ring-specific but HEMC-derived angles
C5    C4    N21   ga_33   ;  corrin ring-specific but HEMC-derived angles
C19   C1    N21   ga_13   ;  corrin ring-specific but HEMC-derived angles
N22   C6    C5    ga_33   ;  corrin ring-specific but HEMC-derived angles
N22   C6    C7    ga_33   ;  corrin ring-specific but HEMC-derived angles
N22   C9    C8    ga_33   ;  corrin ring-specific but HEMC-derived angles
C9    N22   C6    ga_6    ;  corrin ring-specific but HEMC-derived angles
C10   C9    N22   ga_33   ;  corrin ring-specific but HEMC-derived angles
N23   C11   C10   ga_33   ;  corrin ring-specific but HEMC-derived angles
N23   C11   C12   ga_33   ;  corrin ring-specific but HEMC-derived angles
N23   C14   C13   ga_33   ;  corrin ring-specific but HEMC-derived angles
C14   N23   C11   ga_6    ;  corrin ring-specific but HEMC-derived angles
C15   C14   N23   ga_33   ;  corrin ring-specific but HEMC-derived angles
N24   C16   C15   ga_33   ;  corrin ring-specific but HEMC-derived angles
N24   C19   C1    ga_13   ;  corrin ring-specific but HEMC-derived angles
C19   N24   C16   ga_6    ;  corrin ring-specific but HEMC-derived angles
C17   C16   N24   ga_33   ;  corrin ring-specific but HEMC-derived angles
C18   C19   N24   ga_13   ;  corrin ring-specific but HEMC-derived angles
C3R   O2P   P     ga_26   ;  phosphate angle from ADE
O2P   P     O3    ga_5    ;  phosphate angle from ADE
O2P   P     O4    ga_14   ;  phosphate angle from ADE
O2P   P     O5    ga_14   ;  phosphate angle from ADE
O3    P     O4    ga_14   ;  phosphate angle from ADE
O3    P     O5    ga_14   ;  phosphate angle from ADE
O4    P     O5    ga_29   ;  phosphate angle from ADE
P     O3    C2P   ga_26   ;  phosphate angle from ADE
O2P   C3R   C2R   ga_9    ;  phosphate oxygen to CHn carbons from ADE
O2P   C3R   C4R   ga_9    ;  phosphate oxygen to CHn carbons from ADE
O3    C2P   C1P   ga_9    ;  phosphate oxygen to CHn carbons from ADE
O3    C2P   C3P   ga_9    ;  phosphate oxygen to CHn carbons from ADE
C1    C2    C26   ga_13   ;  CHn-CHn-CHn angles
C1    C2    C25   ga_13   ;  CHn-CHn-CHn angles
C1    C2    C3    ga_13   ;  CHn-CHn-CHn angles
C1    C19   C18   ga_13   ;  CHn-CHn-CHn angles
C2    C1    C19   ga_13   ;  CHn-CHn-CHn angles
C2    C1    C20   ga_13   ;  CHn-CHn-CHn angles
C2    C3    C30   ga_13   ;  CHn-CHn-CHn angles
C3    C2    C25   ga_13   ;  CHn-CHn-CHn angles
C3    C2    C26   ga_13   ;  CHn-CHn-CHn angles
C3    C30   C31   ga_13   ;  CHn-CHn-CHn angles
C7    C8    C41   ga_13   ;  CHn-CHn-CHn angles
C8    C41   C42   ga_13   ;  CHn-CHn-CHn angles
C12   C13   C48   ga_13   ;  CHn-CHn-CHn angles
C13   C12   C46   ga_13   ;  CHn-CHn-CHn angles
C13   C12   C47   ga_13   ;  CHn-CHn-CHn angles
C13   C48   C49   ga_13   ;  CHn-CHn-CHn angles
C17   C18   C19   ga_13   ;  CHn-CHn-CHn angles
C17   C18   C60   ga_13   ;  CHn-CHn-CHn angles
C17   C55   C56   ga_13   ;  CHn-CHn-CHn angles
C18   C17   C54   ga_13   ;  CHn-CHn-CHn angles
C18   C17   C55   ga_13   ;  CHn-CHn-CHn angles
C19   C1    C20   ga_13   ;  CHn-CHn-CHn angles
C19   C18   C60   ga_13   ;  CHn-CHn-CHn angles
C25   C2    C26   ga_13   ;  CHn-CHn-CHn angles
C46   C12   C47   ga_13   ;  CHn-CHn-CHn angles
C54   C17   C55   ga_13   ;  CHn-CHn-CHn angles
C36   C7    C37   ga_13   ;  CHn-CHn-CHn angles
C2    C3    C4    ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C4    C3    C30   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C6    C7    C8    ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C6    C7    C36   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C6    C7    C37   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C7    C8    C9    ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C8    C7    C36   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C8    C7    C37   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C9    C8    C41   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C11   C12   C13   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C11   C12   C46   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C11   C12   C47   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C12   C13   C14   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
C14   C13   C48   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
```

```
   C16   C17   C18   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
   C16   C17   C54   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
   C16   C17   C55   ga_15   ;  sp3-sp3-sp2 corrin ring carbon angle
   C4    C5    C6    ga_27   ;  sp2-sp2-sp2 corrin ring carbon angle
   C9    C10   C11   ga_27   ;  sp2-sp2-sp2 corrin ring carbon angle
   C14   C15   C16   ga_27   ;  sp2-sp2-sp2 corrin ring carbon angle
   C3    C4    C5    ga_37   ;  sp3-sp2-sp2 corrin ring carbon angle
   C4    C5    C35   ga_37   ;  sp3-sp2-sp2 corrin ring carbon angle
   C5    C6    C7    ga_37   ;  sp3-sp2-sp2 corrin ring carbon angle
   C6    C5    C35   ga_37   ;  sp3-sp2-sp2 corrin ring carbon angle
   C8    C9    C10   ga_37   ;  sp3-sp2-sp2 corrin ring carbon angle
   C10   C11   C12   ga_37   ;  sp3-sp2-sp2 corrin ring carbon angle
   C13   C14   C15   ga_37   ;  sp3-sp2-sp2 corrin ring carbon angle
   C14   C15   C53   ga_37   ;  sp3-sp2-sp2 corrin ring carbon angle
   C15   C16   C17   ga_37   ;  sp3-sp2-sp2 corrin ring carbon angle
   C16   C15   C53   ga_37   ;  sp3-sp2-sp2 corrin ring carbon angle
   C5M   C5B   C4B   ga_37   ;  sp2-sp2-methyl dimethylbenzimidazole angle
   C5M   C5B   C6B   ga_37   ;  sp2-sp2-methyl dimethylbenzimidazole angle
   C6M   C6B   C5B   ga_37   ;  sp2-sp2-methyl dimethylbenzimidazole angle
   C6M   C6B   C7B   ga_37   ;  sp2-sp2-methyl dimethylbenzimidazole angle
   C4B   C5B   C6B   ga_27   ;  Csp2-Csp2-Csp2 dimethylbenzimidazole angle
   C5B   C4B   C9B   ga_27   ;  Csp2-Csp2-Csp2 dimethylbenzimidazole angle
   C5B   C6B   C7B   ga_27   ;  Csp2-Csp2-Csp2 dimethylbenzimidazole angle
   N1B   C1R   C2R   ga_9    ;  from ADE Nsp2-containing dimethylbenzimidazole angle
   N1B   C1R   O6R   ga_9    ;  from ADE Nsp2-containing dimethylbenzimidazole angle
   N1B   C2B   N3B   ga_7    ;  from ADE Nsp2-containing dimethylbenzimidazole angle
   C2B   N1B   C1R   ga_37   ;  from ADE Nsp2-containing dimethylbenzimidazole angle
   N3B   C9B   C4B   ga_39   ;  from ADE Nsp2-containing dimethylbenzimidazole angle
   C9B   N3B   C2B   ga_7    ;  from ADE Nsp2-containing dimethylbenzimidazole angle
   C1R   C2R   C3R   ga_8    ;  from ADE sugar angle
   C1R   C2R   O7R   ga_9    ;  from ADE sugar angle
   C1R   O6R   C4R   ga_10   ;  from ADE sugar angle
   C2R   C1R   O6R   ga_9    ;  from ADE sugar angle
   C2R   C3R   C4R   ga_8    ;  from ADE sugar angle
   C2R   O7R   HAA   ga_12   ;  from ADE sugar angle
   C3R   C2R   O7R   ga_9    ;  from ADE sugar angle
   C3R   C4R   C5R   ga_8    ;  from ADE sugar angle
   C3R   C4R   O6R   ga_9    ;  from ADE sugar angle
   C4R   C5R   O8R   ga_9    ;  from ADE sugar angle
   C5R   C4R   O6R   ga_9    ;  from ADE sugar angle
   C1P   C2P   C3P   ga_13   ;  amide linkage angle
   C57   C56   C55   ga_13   ;  amide linkage angle
   C57   N59   HAC   ga_23   ;  from GLN angle
   N59   C57   O58   ga_33   ;  from GLN angle
   C56   C57   N59   ga_19   ;  from GLN angle
   O58   C57   C56   ga_30   ;  from GLN angle
   C1P   N59   C57   ga_31   ;  from force field angle definitions
   C1P   N59   HAC   ga_18   ;  from force field angle definitions
   C2P   C1P   N59   ga_15   ;  from force field angle definitions
   C5B   C4B   H4B   ga_25   ;  from PHE C-C-H angle
   C9B   C4B   H4B   ga_25   ;  from PHE C-C-H angle
   C6B   C7B   H7B   ga_25   ;  from PHE C-C-H angle
   C8B   C7B   H7B   ga_25   ;  from PHE C-C-H angle
   N1B   C2B   H2B   ga_36   ;  from ADE N-C-H angle
   N3B   C2B   H2B   ga_36   ;  from ADE N-C-H angle
   C9    C10   H10   ga_25   ;  corrin ring C-C10-H angle
   C11   C10   H10   ga_20   ;  corrin ring C-C10-H angle
   C5R   O8R   N     ga_12   ;  for PHE-B12 bridge (just replaces O8R H atom with CLB)
;
;  standard Phenylalanine angle topology
;
   -C     N     H     ga_32  ;  STANDARD PHE (phenylalanine)
   -C     N    CA     ga_31  ;  STANDARD PHE (phenylalanine)
    H     N    CA     ga_18  ;  STANDARD PHE (phenylalanine)
    N    CA    CB     ga_13  ;  STANDARD PHE (phenylalanine)
    N    CA     C     ga_13  ;  STANDARD PHE (phenylalanine)
   CB    CA     C     ga_13  ;  STANDARD PHE (phenylalanine)
   CA    CB    CG     ga_15  ;  STANDARD PHE (phenylalanine)
   CB    CG   CD1     ga_27  ;  STANDARD PHE (phenylalanine)
   CB    CG   CD2     ga_27  ;  STANDARD PHE (phenylalanine)
  CD1    CG   CD2     ga_27  ;  STANDARD PHE (phenylalanine)
   CG   CD1   HD1     ga_25  ;  STANDARD PHE (phenylalanine)
   CG   CD1   CE1     ga_27  ;  STANDARD PHE (phenylalanine)
```

```
  HD1   CD1   CE1     ga_25  ;  STANDARD PHE (phenylalanine)
   CG   CD2   HD2     ga_25  ;  STANDARD PHE (phenylalanine)
   CG   CD2   CE2     ga_27  ;  STANDARD PHE (phenylalanine)
  HD2   CD2   CE2     ga_25  ;  STANDARD PHE (phenylalanine)
  CD1   CE1   HE1     ga_25  ;  STANDARD PHE (phenylalanine)
  CD1   CE1    CZ     ga_27  ;  STANDARD PHE (phenylalanine)
  HE1   CE1    CZ     ga_25  ;  STANDARD PHE (phenylalanine)
  CD2   CE2   HE2     ga_25  ;  STANDARD PHE (phenylalanine)
  CD2   CE2    CZ     ga_27  ;  STANDARD PHE (phenylalanine)
  HE2   CE2    CZ     ga_25  ;  STANDARD PHE (phenylalanine)
  CE1    CZ   CE2     ga_27  ;  STANDARD PHE (phenylalanine)
  CE1    CZ    HZ     ga_25  ;  STANDARD PHE (phenylalanine)
  CE2    CZ    HZ     ga_25  ;  STANDARD PHE (phenylalanine)
   CA     C     O     ga_30  ;  STANDARD PHE (phenylalanine)
   CA     C    +N     ga_19  ;  STANDARD PHE (phenylalanine)
    O     C    +N     ga_33  ;  STANDARD PHE (phenylalanine)
  O8R   N    CA      ga_33   ;  new OLB-CLB linkage angles
;
; cyano group addition to the original B12 topology
;
   FE    C1N   N1C   ga_41   ;  cyano group from HEMC CO group
   N21   FE    C1N   ga_1    ;  cyano group from HEMC CO group
   N22   FE    C1N   ga_1    ;  cyano group from HEMC CO group
   N23   FE    C1N   ga_1    ;  cyano group from HEMC CO group
   N24   FE    C1N   ga_1    ;  cyano group from HEMC CO group
   N3B   FE    C1N   ga_1   ;  cyano group from HEMC CO group (found during pdb2gmx)

   [ impropers ]
;  ai    aj    ak    al   gromos type
   C1R   C8B   C2B   N1B   gi_1    ;  dimethylbenzimidazole improper for planarity
   N1B   C8B   C9B   N3B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C8B   N1B   C7B   C9B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C8B   N1B   C2B   N3B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C8B   C7B   C6B   C5B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C8B   C9B   N3B   C2B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C7B   C8B   C9B   C4B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C7B   C6B   C5B   C4B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C6B   C5B   C4B   C9B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C5B   C4B   C9B   C8B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C9B   C8B   C7B   C6B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C9B   C4B   N3B   C8B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C9B   N3B   C2B   N1B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C2B   N1B   C8B   C9B   gi_1    ;  dimethylbenzimidazole improper for planarity
   C5M   C4B   C6B   C5B   gi_1    ;  dimethylbenzimidazole methyl groups
   C6M   C7B   C5B   C6B   gi_1    ;  dimethylbenzimidazole methyl groups
   C2B   N1B   N3B   H2B   gi_1    ;  dimethylbenzimidazole H atom-containing planarity
   C4B   C9B   C5B   H4B   gi_1    ;  dimethylbenzimidazole H atom-containing planarity
   C7B   C8B   C6B   H7B   gi_1    ;  dimethylbenzimidazole H atom-containing planarity
   N3B   FE    C2B   C9B   gi_1    ;  Fe-dimethylbenzimidazole planarity
   N21   C4    C5    C6    gi_1    ;  HEMC-based conjugated corrin ring improper planarity
   N22   C6    C5    C4    gi_1    ;  HEMC-based conjugated corrin ring improper planarity
   N22   C9    C10   C11   gi_1    ;  HEMC-based conjugated corrin ring improper planarity
   N23   C11   C10   C9    gi_1    ;  HEMC-based conjugated corrin ring improper planarity
   N23   C14   C15   C16   gi_1    ;  HEMC-based conjugated corrin ring improper planarity
   N24   C16   C15   C14   gi_1    ;  HEMC-based conjugated corrin ring improper planarity
   C5    C4    C6    C35   gi_1    ;  corrin ring on-Csp2 methyl group improper planarity
   C15   C16   C14   C53   gi_1    ;  corrin ring on-Csp2 methyl group improper planarity
   C27   O28   N29   C26   gi_1    ;  from GLN improper torsion definition
   N29   HAE   HAD   C27   gi_1    ;  from GLN improper torsion definition
   C32   O34   N33   C31   gi_1    ;  from GLN improper torsion definition
   N33   HAG   HAF   C32   gi_1    ;  from GLN improper torsion definition
   C38   O39   N40   C37   gi_1    ;  from GLN improper torsion definition
   N40   HAI   HAH   C38   gi_1    ;  from GLN improper torsion definition
   C43   O44   N45   C42   gi_1    ;  from GLN improper torsion definition
   N45   HAK   HAJ   C43   gi_1    ;  from GLN improper torsion definition
   C50   O51   N52   C49   gi_1    ;  from GLN improper torsion definition
   N52   HAM   HAL   C50   gi_1    ;  from GLN improper torsion definition
   C61   O63   N62   C60   gi_1    ;  from GLN improper torsion definition
   N62   HAO   HAN   C61   gi_1    ;  from GLN improper torsion definition
   C57   C56   O58   N59   gi_1    ;  amide linkage C57 planarity
   N59   C57   C1P   HAC   gi_1    ;  amide linkage N59 planarity
   C4    N21   C3    C5    gi_1    ;  corrin ring C sp2 planarity
   C5    C4    C6    C35   gi_1    ;  corrin ring C sp2 planarity
```

```
   C6    N22   C5    C7    gi_1    ;  corrin ring C sp2 planarity
   C9    N22   C10   C8    gi_1    ;  corrin ring C sp2 planarity
   C11   N23   C10   C12   gi_1    ;  corrin ring C sp2 planarity
   C14   N23   C15   C13   gi_1    ;  corrin ring C sp2 planarity
   C15   C14   C16   C53   gi_1    ;  corrin ring C sp2 planarity
   C16   N24   C15   C17   gi_1    ;  corrin ring C sp2 planarity
   C10   C9    C11   H10   gi_1    ;  corrin ring C10 planarity
   C18   C17   C19   C60   gi_2    ;  corrin ring C atom sp3 tetrahedral geometry
   C19   N24   C18   C1    gi_2    ;  corrin ring C atom sp3 tetrahedral geometry
   C3    C2    C30   C4    gi_2    ;  corrin ring C atom sp3 tetrahedral geometry
   C8    C9    C7    C41   gi_2    ;  corrin ring C atom sp3 tetrahedral geometry
   C13   C14   C12   C48   gi_2    ;  corrin ring C atom sp3 tetrahedral geometry
   C2P   C1P   O3    C3P   gi_2    ;  corrin ring C atom sp3 tetrahedral geometry
   C1R   N1B   C2R   O6R   gi_2    ;  sugar C atom tetrahedral geometry
   C2R   O7R   C3R   C1R   gi_2    ;  sugar C atom tetrahedral geometry
   C3R   C5R   O6R   C4R   gi_2    ;  sugar C atom tetrahedral geometry
   C3R   C2R   O2P   C4R   gi_2    ;  sugar C atom tetrahedral geometry
   FE    C1    C4    N21   gi_3    ;  HEMC improper definition
   FE    C6    C9    N22   gi_3    ;  HEMC improper definition
   FE    C11   C14   N23   gi_3    ;  HEMC improper definition
   FE    C16   C19   N24   gi_3    ;  HEMC improper definition
    N    -C    CA     H    gi_1    ;  PHE improper definition
   CA     N     C    CB    gi_2    ;  PHE improper definition
   CG   CD1   CD2    CB    gi_1    ;  PHE improper definition
   CG   CD1   CE1    CZ    gi_1    ;  PHE improper definition
   CG   CD2   CE2    CZ    gi_1    ;  PHE improper definition
  CD1    CG   CD2   CE2    gi_1    ;  PHE improper definition
  CD1    CG   CE1   HD1    gi_1    ;  PHE improper definition
  CD1   CE1    CZ   CE2    gi_1    ;  PHE improper definition
  CD2    CG   CD1   CE1    gi_1    ;  PHE improper definition
  CD2    CG   CE2   HD2    gi_1    ;  PHE improper definition
  CD2   CE2    CZ   CE1    gi_1    ;  PHE improper definition
  HE1   CD1    CZ   CE1    gi_1    ;  PHE improper definition
  HE2   CD2    CZ   CE2    gi_1    ;  PHE improper definition
   CZ   CE1   CE2    HZ    gi_1    ;  PHE improper definition
    C    CA    +N     O    gi_1    ;  PHE improper definition

 [ dihedrals ]
;  ai    aj    ak    al   gromos type
   C3R   O2P   P     O3    gd_20   ;  from ADE phosphate definition
   C3R   O2P   P     O3    gd_27   ;  from ADE phosphate definition
   C2P   O3    P     O2P   gd_20   ;  from ADE phosphate definition
   C2P   O3    P     O2P   gd_27   ;  from ADE phosphate definition
   P     O3    C2P   C1P   gd_7    ;  from ADE phosphate definition
   C2R   C3R   O2P   P     gd_29   ;  from ADE phosphate definition
   O8R   C5R   C4R   O6R   gd_8    ;  from ADE sugar dihedrals
   O8R   C5R   C4R   O6R   gd_25   ;  from ADE sugar dihedrals
   O8R   C5R   C4R   C3R   gd_17   ;  from ADE sugar dihedrals
   O8R   C5R   C4R   C3R   gd_34   ;  from ADE sugar dihedrals
   C3R   C4R   O6R   C1R   gd_29   ;  from ADE sugar dihedrals
   C5R   C4R   C3R   C2R   gd_34   ;  from ADE sugar dihedrals
   C5R   C4R   C3R   O2P   gd_17   ;  from ADE sugar dihedrals
   O6R   C4R   C3R   C2R   gd_17   ;  from ADE sugar dihedrals
   O6R   C4R   C3R   O2P   gd_18   ;  from ADE sugar dihedrals
   C4R   O6R   C1R   C2R   gd_29   ;  from ADE sugar dihedrals
   O6R   C1R   C2R   O7R   gd_18   ;  from ADE sugar dihedrals
   O6R   C1R   C2R   C3R   gd_17   ;  from ADE sugar dihedrals
   O6R   C1R   C2R   C3R   gd_34   ;  from ADE sugar dihedrals
   C1R   C2R   C3R   C4R   gd_34   ;  from ADE sugar dihedrals
   C1R   C2R   C3R   O2P   gd_17   ;  from ADE sugar dihedrals
   O7R   C2R   C3R   C4R   gd_17   ;  from ADE sugar dihedrals
   O7R   C2R   C3R   O2P   gd_18   ;  from ADE sugar dihedrals
   C1R   C2R   O7R   HAA   gd_23   ;  from ADE sugar dihedrals
   N1B   C1R   C2R   O7R   gd_17   ;  from ADE sugar dihedrals
   O6R   C1R   N1B   C8B   gd_16   ;  from ADE sugar dihedrals
   N1B   C1R   C2R   C3R   gd_17   ;  additional ADE-based sugar dihedral
   N1B   C1R   O6R   C4R   gd_17   ;  additional ADE-based sugar dihedral
   C2    C26   C27   N29   gd_40   ;  from GLN dihedral
   C26   C27   N29   HAD   gd_14   ;  from GLN dihedral
   C30   C31   C32   N33   gd_40   ;  from GLN dihedral
   C31   C32   N33   HAF   gd_14   ;  from GLN dihedral
   C7    C37   C38   N40   gd_40   ;  from GLN dihedral
   C37   C38   N40   HAH   gd_14   ;  from GLN dihedral
```

```
C41   C42   C43   N45   gd_40   ;  from GLN dihedral
C42   C43   N45   HAJ   gd_14   ;  from GLN dihedral
C48   C49   C50   N52   gd_40   ;  from GLN dihedral
C49   C50   N52   HAL   gd_14   ;  from GLN dihedral
C18   C60   C61   N62   gd_40   ;  from GLN dihedral
C60   C61   N62   HAN   gd_14   ;  from GLN dihedral
C5M   C5B   C6B   C6M   gd_33   ;  dimethylbenzimidazole methyl dihedral fix
C4B   C5B   C6B   C6M   gd_10   ;  dimethylbenzimidazole methyl dihedral fix
C8B   C7B   C6B   C6M   gd_10   ;  dimethylbenzimidazole methyl dihedral fix
C7B   C6B   C5B   C5M   gd_10   ;  dimethylbenzimidazole methyl dihedral fix
C9B   C4B   C5B   C5M   gd_10   ;  dimethylbenzimidazole methyl dihedral fix
C35   C5    C4    N21   gd_10   ;  corrin ring methyl planarity
C35   C5    C6    N22   gd_10   ;  corrin ring methyl planarity
C53   C15   C14   N23   gd_10   ;  corrin ring methyl planarity
C53   C1    C16   N24   gd_10   ;  corrin ring methyl planarity
N24   C19   C1    N21   gd_34   ;  corrin ring but HEMC-derived N,C torsions
N24   C19   C1    N21   gd_34   ;  corrin ring but HEMC-derived N,C torsions
N21   C4    C5    C6    gd_15   ;  corrin ring but HEMC-derived N,C torsions
C4    C5    C6    N22   gd_15   ;  corrin ring but HEMC-derived N,C torsions
N22   C9    C10   C11   gd_15   ;  corrin ring but HEMC-derived N,C torsions
C9    C10   C11   N23   gd_15   ;  corrin ring but HEMC-derived N,C torsions
N23   C14   C15   C16   gd_15   ;  corrin ring but HEMC-derived N,C torsions
C14   C15   C16   N24   gd_15   ;  corrin ring but HEMC-derived N,C torsions
C1    C2    C26   C27   gd_40   ;  corrin ring HEMC derived CHn torsions
C25   C2    C26   C27   gd_34   ;  corrin ring HEMC derived CHn torsions
C2    C26   C27   O28   gd_34   ;  corrin ring HEMC derived CHn torsions
C2    C3    C30   C31   gd_9    ;  corrin ring HEMC derived CHn torsions
C3    C30   C31   C32   gd_34   ;  corrin ring HEMC derived CHn torsions
C30   C31   C32   O34   gd_40   ;  corrin ring HEMC derived CHn torsions
C6    C7    C37   C38   gd_40   ;  corrin ring HEMC derived CHn torsions
C36   C7    C37   C38   gd_40   ;  corrin ring HEMC derived CHn torsions
C7    C37   C38   O39   gd_40   ;  corrin ring HEMC derived CHn torsions
C7    C8    C41   C42   gd_9    ;  corrin ring HEMC derived CHn torsions
C8    C41   C42   C43   gd_34   ;  corrin ring HEMC derived CHn torsions
C41   C42   C43   O44   gd_40   ;  corrin ring HEMC derived CHn torsions
C12   C13   C48   C49   gd_9    ;  corrin ring HEMC derived CHn torsions
C13   C48   C49   C50   gd_34   ;  corrin ring HEMC derived CHn torsions
C48   C49   C50   O51   gd_40   ;  corrin ring HEMC derived CHn torsions
C16   C17   C55   C56   gd_40   ;  corrin ring HEMC derived CHn torsions
C54   C17   C56   C57   gd_40   ;  corrin ring HEMC derived CHn torsions
C17   C55   C56   C57   gd_34   ;  corrin ring HEMC derived CHn torsions
C55   C56   C57   O58   gd_40   ;  corrin ring HEMC derived CHn torsions
C17   C18   C60   C61   gd_9    ;  corrin ring HEMC derived CHn torsions
C18   C60   C61   O63   gd_34   ;  corrin ring HEMC derived CHn torsions
C18   C17   C55   C56   gd_9    ;  corrin ring HEMC derived CHn torsions
N59   C1P   C2P   O3    gd_34   ;  amide linkage torsion terms
N59   C1P   C2P   C3P   gd_34   ;  amide linkage torsion terms
C2P   C1P   N59   C57   gd_34   ;  amide linkage torsion terms
C56   C57   N59   C1P   gd_14   ;  amide linkage torsion terms
C55   C56   C57   N59   gd_40   ;  amide linkage torsion terms
C17   C55   C56   C57   gd_40   ;  amide linkage torsion terms
C5    C4    N21   C1    gd_14   ;  corrin ring sp3-containing torsions
C10   C9    N22   C6    gd_14   ;  corrin ring sp3-containing torsions
C5    C6    N22   C9    gd_14   ;  corrin ring sp3-containing torsions
C15   C14   N23   C11   gd_14   ;  corrin ring sp3-containing torsions
C10   C11   N23   C14   gd_14   ;  corrin ring sp3-containing torsions
C15   C16   N24   C19   gd_14   ;  corrin ring sp3-containing torsions
C4    N21   C1    C2    gd_40   ;  corrin ring sp3-containing torsions
C18   C19   N24   C16   gd_40   ;  corrin ring sp3-containing torsions
N22   C6    C5    C4    gd_33   ;  corrin ring sp3-containing torsions
N23   C11   C10   C9    gd_33   ;  corrin ring sp3-containing torsions
C19   C18   C17   C55   gd_34   ;  corrin ring sp3-containing torsions
C55   C17   C16   C15   gd_40   ;  corrin ring sp3-containing torsions
C17   C18   C19   C1    gd_34   ;  corrin ring sp3-containing torsions
C18   C19   C1    C2    gd_34   ;  corrin ring sp3-containing torsions
C17   C16   C15   C14   gd_10   ;  corrin ring sp3-containing torsions
C16   C15   C14   C13   gd_10   ;  corrin ring sp3-containing torsions
C15   C14   C13   C12   gd_40   ;  corrin ring sp3-containing torsions
C6    C7    C8    C9    gd_34   ;  corrin ring sp3-containing torsions
C8    C7    C6    C5    gd_34   ;  corrin ring sp3-containing torsions
C30   C3    C2    C1    gd_34   ;  corrin ring sp3-containing torsions
C11   C12   C13   C14   gd_34   ;  corrin ring sp3-containing torsions
C13   C12   C11   C10   gd_34   ;  corrin ring sp3-containing torsions
```

```
 C10   C9    C8    C7    gd_10   ;  corrin ring sp3-containing torsions
 C5    C4    C3    C30   gd_34   ;  corrin ring sp3-containing torsions
 C3    C2    C1    C19   gd_34   ;  corrin ring sp3-containing torsions
-CA    -C     N    CA    gd_14   ;  PHE dihedral definition
 -C     N    CA     C    gd_39      ;  PHE dihedral definition
  N    CA    CB    CG    gd_34      ;  PHE dihedral definition
  N    CA     C    +N    gd_40      ;  PHE dihedral definition
 CA    CB    CG   CD1    gd_40      ;  PHE dihedral definition
 C4R   C5R   O8R   N     gd_23   ;  LYS-B12 linkage dihedral terms
 C5R   O8R   N    CA     gd_10   ;  LYS-B12 linkage dihedral terms
 O8R     N   CA    C     gd_10   ;  LYS-B12 linkage dihedral terms
 O8R     N   CA   CB     gd_10   ;  LYS-B12 linkage dihedral terms
```

# GROMOS96 Charge Groups for B1-linked $B_{12}$-Insulin

Format below is GROMACS ffG53a6.top file-ready.

```
   208          C     22     PCB     C27      87        0.29     12.011   ; qtot -1.71
   209          O     22     PCB     O28      87       -0.45    15.9994   ; qtot -2.16
   210         NT     22     PCB     N29      87       -0.72    14.0067   ; qtot -2.88
   211          H     22     PCB     HAE      87        0.44      1.008   ; qtot -2.44
   212          H     22     PCB     HAD      87        0.44      1.008   ; qtot -2
   213          C     22     PCB     C32      88        0.29     12.011   ; qtot -1.71
   214          O     22     PCB     O34      88       -0.45    15.9994   ; qtot -2.16
   215         NT     22     PCB     N33      88       -0.72    14.0067   ; qtot -2.88
   216          H     22     PCB     HAG      88        0.44      1.008   ; qtot -2.44
   217          H     22     PCB     HAF      88        0.44      1.008   ; qtot -2
   218          C     22     PCB     C38      89        0.29     12.011   ; qtot -1.71
   219          O     22     PCB     O39      89       -0.45    15.9994   ; qtot -2.16
   220         NT     22     PCB     N40      89       -0.72    14.0067   ; qtot -2.88
   221          H     22     PCB     HAI      89        0.44      1.008   ; qtot -2.44
   222          H     22     PCB     HAH      89        0.44      1.008   ; qtot -2
   223          C     22     PCB     C43      90        0.29     12.011   ; qtot -1.71
   224          O     22     PCB     O44      90       -0.45    15.9994   ; qtot -2.16
   225         NT     22     PCB     N45      90       -0.72    14.0067   ; qtot -2.88
   226          H     22     PCB     HAK      90        0.44      1.008   ; qtot -2.44
   227          H     22     PCB     HAJ      90        0.44      1.008   ; qtot -2
   228          C     22     PCB     C50      91        0.29     12.011   ; qtot -1.71
   229          O     22     PCB     O51      91       -0.45    15.9994   ; qtot -2.16
   230         NT     22     PCB     N52      91       -0.72    14.0067   ; qtot -2.88
   231          H     22     PCB     HAM      91        0.44      1.008   ; qtot -2.44
   232          H     22     PCB     HAL      91        0.44      1.008   ; qtot -2
   233          C     22     PCB     C61      92        0.29     12.011   ; qtot -1.71
   234          O     22     PCB     O63      92       -0.45    15.9994   ; qtot -2.16
   235         NT     22     PCB     N62      92       -0.72    14.0067   ; qtot -2.88
   236          H     22     PCB     HAO      92        0.44      1.008   ; qtot -2.44
   237          H     22     PCB     HAN      92        0.44      1.008   ; qtot -2
   238        CH3     22     PCB     C20      93           0     15.035   ; qtot -2
   239        CH3     22     PCB     C25      94           0     15.035   ; qtot -2
   240        CH3     22     PCB     C36      95           0     15.035   ; qtot -2
   241        CH3     22     PCB     C46      96           0     15.035   ; qtot -2
   242        CH3     22     PCB     C47      97           0     15.035   ; qtot -2
   243        CH3     22     PCB     C54      98           0     15.035   ; qtot -2
   244        CH2     22     PCB     C26      99           0     14.027   ; qtot -2
   245        CH2     22     PCB     C30     100           0     14.027   ; qtot -2
   246        CH2     22     PCB     C31     100           0     14.027   ; qtot -2
   247        CH2     22     PCB     C37     101           0     14.027   ; qtot -2
   248        CH2     22     PCB     C41     102           0     14.027   ; qtot -2
   249        CH2     22     PCB     C42     102           0     14.027   ; qtot -2
   250        CH2     22     PCB     C48     103           0     14.027   ; qtot -2
   251        CH2     22     PCB     C49     103           0     14.027   ; qtot -2
   252        CH2     22     PCB     C56     104           0     14.027   ; qtot -2
   253        CH2     22     PCB     C55     104           0     14.027   ; qtot -2
   254        CH2     22     PCB     C60     105           0     14.027   ; qtot -2
   255        CH1     22     PCB     C2R     106        0.15     13.019   ; qtot -1.85
   256         OA     22     PCB     O7R     106      -0.548    15.9994   ; qtot -2.398
   257          H     22     PCB     HAA     106       0.398      1.008   ; qtot -2
   258        CH1     22     PCB     C1R     107         0.2     13.019   ; qtot -1.8
   259         OA     22     PCB     O6R     107       -0.36    15.9994   ; qtot -2.16
   260        CH1     22     PCB     C4R     107        0.16     13.019   ; qtot -2
   261        CH1     22     PCB     C3R     108           0     13.019   ; qtot -2
   262        CH2     22     PCB     C5R     109        0.15     14.027   ; qtot -1.85
   263         OA     22     PCB     O8R     109       -0.15    15.9994   ; qtot -2
   264         NL     22     PCB       N     109       -0.66    14.0067   ; qtot -2.66
   265          H     22     PCB      H1     109        0.44      1.008   ; qtot -2.22
   266          H     22     PCB      H2     109        0.44      1.008   ; qtot -1.78
   267          P     22     PCB       P     110           1    30.9738   ; qtot -0.78
   268         OA     22     PCB     O2P     110      -0.365    15.9994   ; qtot -1.145
   269         OM     22     PCB      O4     110      -0.635    15.9994   ; qtot -1.78
   270         OM     22     PCB      O5     110      -0.635    15.9994   ; qtot -2.415
   271         OA     22     PCB      O3     110      -0.365    15.9994   ; qtot -2.78
   272        CH1     22     PCB     C2P     111           0     13.019   ; qtot -2.78
   273        CH3     22     PCB     C3P     111           0     15.035   ; qtot -2.78
   274        CH2     22     PCB     C1P     111           0     14.027   ; qtot -2.78
   275          N     22     PCB     N59     112        -0.3     14.0067   ; qtot -3.08
```

```
276          H     22    PCB    HAC    112       0.44      1.008   ; qtot -2.64
277          C     22    PCB    C57    112       0.19     12.011   ; qtot -2.45
278          O     22    PCB    O58    112      -0.33    15.9994   ; qtot -2.78
279          C     22    PCB    C5B    113          0     12.011   ; qtot -2.78
280        CH3     22    PCB    C5M    113          0     15.035   ; qtot -2.78
281          C     22    PCB    C6B    114          0     12.011   ; qtot -2.78
282        CH3     22    PCB    C6M    114          0     15.035   ; qtot -2.78
283          C     22    PCB    C4B    115      -0.14     12.011   ; qtot -2.92
284          H     22    PCB    H4B    115       0.14      1.008   ; qtot -2.78
285          C     22    PCB    C7B    116      -0.14     12.011   ; qtot -2.92
286          H     22    PCB    H7B    116       0.14      1.008   ; qtot -2.78
287         NR     22    PCB    N3B    117       -0.2    14.0067   ; qtot -2.98
288          C     22    PCB    C9B    117        0.2     12.011   ; qtot -2.78
289         NR     22    PCB    N1B    118       -0.2    14.0067   ; qtot -2.98
290          C     22    PCB    C8B    118        0.2     12.011   ; qtot -2.78
291          C     22    PCB    C2B    119       0.14     12.011   ; qtot -2.64
292          H     22    PCB    H2B    119      -0.14      1.008   ; qtot -2.78
293          C     22    PCB     C5    120          0     12.011   ; qtot -2.78
294        CH3     22    PCB    C35    120          0     15.035   ; qtot -2.78
295          C     22    PCB    C15    121          0     12.011   ; qtot -2.78
296        CH3     22    PCB    C53    121          0     15.035   ; qtot -2.78
297         FE     22    PCB     FE    122        0.6     55.847   ; qtot -2.18
298         NR     22    PCB    N21    122       -0.2    14.0067   ; qtot -2.38
299         NR     22    PCB    N22    122       -0.1    14.0067   ; qtot -2.48
300         NR     22    PCB    N23    122       -0.1    14.0067   ; qtot -2.58
301         NR     22    PCB    N24    122       -0.2    14.0067   ; qtot -2.78
302        CH0     22    PCB     C1    123          0     12.011   ; qtot -2.78
303        CH0     22    PCB     C2    123          0     12.011   ; qtot -2.78
304        CH1     22    PCB     C3    123          0     13.019   ; qtot -2.78
305          C     22    PCB     C4    123          0     12.011   ; qtot -2.78
306          C     22    PCB     C6    124          0     12.011   ; qtot -2.78
307        CH0     22    PCB     C7    124          0     12.011   ; qtot -2.78
308        CH1     22    PCB     C8    124          0     13.019   ; qtot -2.78
309          C     22    PCB     C9    124          0     12.011   ; qtot -2.78
310          C     22    PCB    C16    125          0     12.011   ; qtot -2.78
311        CH0     22    PCB    C17    125          0     12.011   ; qtot -2.78
312        CH1     22    PCB    C18    125          0     13.019   ; qtot -2.78
313        CH1     22    PCB    C19    125          0     13.019   ; qtot -2.78
314          C     22    PCB    C11    126          0     12.011   ; qtot -2.78
315        CH0     22    PCB    C12    126          0     12.011   ; qtot -2.78
316        CH1     22    PCB    C13    126          0     13.019   ; qtot -2.78
317          C     22    PCB    C14    126          0     12.011   ; qtot -2.78
318          C     22    PCB    C10    127      -0.14     12.011   ; qtot -2.92
319          H     22    PCB    H10    127       0.14      1.008   ; qtot -2.78
320        CH1     22    PCB     CA    128      -0.22     13.019   ; qtot -3
321        CH2     22    PCB     CB    129          0     14.027   ; qtot -3
322          C     22    PCB     CG    129          0     12.011   ; qtot -3
323          C     22    PCB    CD1    130      -0.14     12.011   ; qtot -3.14
324         HC     22    PCB    HD1    130       0.14      1.008   ; qtot -3
325          C     22    PCB    CD2    131      -0.14     12.011   ; qtot -3.14
326         HC     22    PCB    HD2    131       0.14      1.008   ; qtot -3
327          C     22    PCB    CE1    132      -0.14     12.011   ; qtot -3.14
328         HC     22    PCB    HE1    132       0.14      1.008   ; qtot -3
329          C     22    PCB    CE2    133      -0.14     12.011   ; qtot -3.14
330         HC     22    PCB    HE2    133       0.14      1.008   ; qtot -3
331          C     22    PCB     CZ    134      -0.14     12.011   ; qtot -3.14
332         HC     22    PCB     HZ    134       0.14      1.008   ; qtot -3
333          C     22    PCB      C    135       0.45     12.011   ; qtot -2.55
334          O     22    PCB      O    135      -0.45    15.9994   ; qtot -3
335          C     22    PCB    C1N    136          0     12.011   ; qtot -3
336          N     22    PCB    N1C    136          0    14.0067   ; qtot -3
```

## References


1. A. Petrus, A.Vortherms, T. Fairchild, and R. Doyle, *ChemMedChem* 2007, **2**, 1717.
2. C. Sundararajan, T. Besanger, R. Labiris, J. Valliant *et. al*., *J Med Chem* 2010, **53**, 2612.
3. B. Hess, C. Kutzner, D. van der Spoel, and E. Lindahl, *J Chem Theory Comput* 2008, **4**, 435.
4. W. van Gunsteren, S. Billeter, A. Eising, I. Tironi *et. al.*, Biomolecular Simulation: The Gromos96 Manual and User Guide, Vdf Hochschulverlag AG, ETH Zürich, 1996, p. 1044.
5. H. Berendsen, J. Postma, W. van Gunsteren, A. DiNola, and J. Haak, *J Chem Phys* 1984, **81**, 3684.
6. T. Darden, D. York, and L. Pedersen, *J Chem Phys* 1993, **98**, 10089.
7. D. Allis, T. Fairchild, and R. Doyle, *Mol BioSyst* 2010, **6**, 1611.
8. J. Wuerges, G. Garau, S. Geremia, S. N. Fedosov, T. E. Petersen, and L. Randaccio, *Proc Natl Acad Sci USA* 2006, **103**, 4386.
9. A. Becke, *J Chem Phys* 1993, **98**, 5648.; P. Stephens, F. Devlin, C. Chabalowski, and M. Frisch, *J Phys Chem* 1994, **98**, 623.
10. W. Hehre, R. Ditchfield, and J. Pople, *J Chem Phys* 1972, **56**, 2257.
11. M. Frisch et al., Gaussian 03, Revision C.02, 2004, Gaussian Inc.: Wallingford, CT (USA).
12. W. Humphrey, A. Dalke, and K. Schulten, *J Molec Graphics* 1996, **14**, 33.