Contents

Preface ix

1 Introduction

1.1 The Development of High-Resolution NMR 1
1.2 Modern High-Resolution NMR and This Book 3
1.2.1 What This Book Contains 4
1.2.2 Pulse Sequence Nomenclature 5
1.3 Applying Modern NMR Techniques 7
References 10

2 Introducing High-Resolution NMR

2.1 Nuclear Spin and Resonance 11
2.2 The Vector Model of NMR 14
2.2.1 The Rotating Frame of Reference 14
2.2.2 Pulses 15
2.2.3 Chemical Shifts and Couplings 17
2.2.4 Spin-Echoes 18
2.3 Time and Frequency Domains 20
2.4 Spin Relaxation 22
2.4.1 Longitudinal Relaxation: Establishing Equilibrium 22
2.4.2 Measuring T_1 with the Inversion Recovery Sequence 24
2.4.3 Transverse Relaxation: Loss of Magnetisation in the x–y Plane 26
2.4.4 Measuring T_2 with a Spin-Echo Sequence 27
2.5 Mechanisms for Relaxation 31
2.5.1 The Path to Relaxation 32
2.5.2 Dipole–Dipole Relaxation 33
2.5.3 Chemical Shift Anisotropy Relaxation 34
2.5.4 Spin Rotation Relaxation 35
2.5.5 Quadrupolar Relaxation 35
2.6 Dynamic Effects in NMR 38
2.6.1 The Influence of Dynamic Exchange 39
2.6.2 Lineshape Analysis and Thermodynamic Parameters 53
2.6.3 Magnetisation Transfer under Slow-Exchange Conditions 55
References 58

3 Practical Aspects of High-Resolution NMR

3.1 An Overview of the NMR Spectrometer 61
3.2 Data Acquisition and Processing 64
3.2.1 Pulse Excitation 64
3.2.2 Signal Detection 66
3.2.3 Sampling the FID 67
3.2.4 Quadrature Detection 73
3.2.5 Phase Cycling 78
3.2.6 Dynamic Range and Signal Averaging 80
3.2.7 Window Functions 83
3.2.8 Phase Correction 88
3.3 Preparing the Sample 89
3.3.1 Selecting the Solvent 89
3.3.2 Reference Compounds 91
3.3.3 Tubes and Sample Volumes 92
3.3.4 Filtering and Degassing 94
3.4 Preparing the Spectrometer 95
3.4.1 The Probe 95
3.4.2 Probe Design and Sensitivity 97
3.4.3 Tuning the Probe 103
3.4.4 The Field Frequency Lock 105
3.4.5 Optimising Field Homogeneity: Shimming 107
3.4.6 Reference Deconvolution 112
3.5 Spectrometer Calibrations 113
3.5.1 Radiofrequency Pulses 113
3.5.2 Pulsed Field Gradients 122
3.5.3 Sample Temperature 124
3.6 Spectrometer Performance Tests 126
3.6.1 Lineshape and Resolution 127
3.6.2 Sensitivity 128
3.6.3 Solvent Presaturation 130
References 130

4 One-Dimensional Techniques

4.1 Single-Pulse Experiment 133
- 4.1.1 Optimising Sensitivity 133
- 4.1.2 Quantitative NMR Measurements and Integration 137
- 4.1.3 Quantification with an Electronic Calibrant: ERETIC 140
- 4.1.4 Quantification with an External Calibrant: PULCON 142

4.2 Spin-Decoupling Methods 143
- 4.2.1 Basis of Spin Decoupling 143
- 4.2.2 Homonuclear Decoupling 143
- 4.2.3 Heteronuclear Decoupling 145

4.3 Spectrum Editing with Spin-Echoes 148
- 4.3.1 J-Modulated Spin-Echo 149
- 4.3.2 APT 152

4.4 Sensitivity Enhancement and Spectrum Editing 153
- 4.4.1 Polarisation Transfer 154
- 4.4.2 INEPT 156
- 4.4.3 DEPT 162
- 4.4.4 DEPTQ 165

4.5 Observing Quadrupolar Nuclei 167

References 168

5 Introducing Two-Dimensional and Pulsed Field Gradient NMR

5.1 Two-Dimensional Experiments 172
- 5.1.1 Generating the Second Dimension 172
- 5.1.2 Correlating Coupled Spins 176

5.2 Practical Aspects of 2D NMR 177
- 5.2.1 Two-Dimensional Lineshapes and Quadrature Detection 177
- 5.2.2 Axial Peaks 181
- 5.2.3 Instrumental Artefacts 182
- 5.2.4 Two-Dimensional Data Acquisition 183
- 5.2.5 Two-Dimensional Data Processing 186

5.3 Coherence and Coherence Transfer 188
- 5.3.1 Coherence Transfer Pathways 190

5.4 Gradient-Selected Spectroscopy 191
- 5.4.1 Signal Selection with Pulsed Field Gradients 192
- 5.4.2 Phase-Sensitive Experiments: *Echo–Antiecho* Selection 195
- 5.4.3 Pulsed Field Gradients in High-Resolution NMR 196
- 5.4.4 Practical Implementation of Pulsed Field Gradients 198
- 5.4.5 Fast Data Acquisition: Single-Scan Two-Dimensional NMR 199

References 201

6 Correlations Through the Chemical Bond I: Homonuclear Shift Correlation

6.1 Correlation Spectroscopy: COSY 203
- 6.1.1 Interpreting COSY 204
- 6.1.2 Peak Fine Structure 207
- 6.1.3 Which COSY Approach? 210
- 6.1.4 COSY-β 211
- 6.1.5 Double-Quantum Filtered COSY (DQF-COSY) 212
- 6.1.6 Long-Range COSY: Detecting Small Couplings 219
- 6.1.7 Relayed-COSY 220

6.2 Total Correlation Spectroscopy: TOCSY 220
- 6.2.1 The TOCSY Sequence 221
- 6.2.2 Applying TOCSY 223
- 6.2.3 Implementing TOCSY 225
- 6.2.4 One-Dimensional TOCSY 227

6.3 Correlating Dilute Spins: INADEQUATE 230
- 6.3.1 Two-Dimensional INADEQUATE 230
- 6.3.2 One-Dimensional INADEQUATE 233
- 6.3.3 Implementing INADEQUATE 233

6.4 Correlating Dilute Spins Via Protons: ADEQUATE 235
- 6.4.1 Two-Dimensional ADEQUATE 236
- 6.4.2 Enhancements to ADEQUATE 237

References 240

7 Correlations Through the Chemical Bond II: Heteronuclear Shift Correlation

7.1 Introduction 243

7.2 Sensitivity 244

7.3 Heteronuclear Single-Bond Correlations 246
- 7.3.1 Heteronuclear Single-Quantum Correlation 246
- 7.3.2 Hybrid HSQC Experiments 253
- 7.3.3 Heteronuclear Multiple-Quantum Correlation 257

7.4 Heteronuclear Multiple-Bond Correlations 261
- 7.4.1 HMBC Sequence 263
- 7.4.2 Applying HMBC 264
- 7.4.3 HMBC Extensions and Variants 266
- 7.4.4 H2BC: Differentiating $^{2}J_{CH}$ and $^{3}J_{CH}$ HMBC Correlations 274
- 7.4.5 Measuring Long-Range $^{n}J_{XH}$ Coupling Constants 275
- 7.4.6 Long-Range HSQMBC: Interrogating Proton-Sparse Molecules 281

7.5 Heteronuclear X-Detected Correlations 282
- 7.5.1 Single-Bond Heteronuclear Correlations 283
- 7.5.2 Multiple-Bond Correlations and Small Couplings 285

7.6 Heteronuclear X–Y Correlations 286
7.6.1 Direct X–Y Correlations 286
7.6.2 Indirect ^{1}H-Detected X–Y Correlations 288
7.7 Parallel Acquisition NMR with Multiple Receivers 291
References 292

8 Separating Shifts and Couplings: J-Resolved and Pure Shift Spectroscopy
8.1 Introduction 295
8.2 Heteronuclear J-Resolved Spectroscopy 295
8.2.1 Measuring Long-Range Proton–Carbon Coupling Constants 298
8.2.2 Practical Considerations 300
8.3 Homonuclear J-Resolved Spectroscopy 301
8.3.1 Tilting, Projections and Symmetrisation 302
8.3.2 Applications 303
8.4 'Indirect' Homonuclear J-Resolved Spectroscopy 304
8.5 Pure Shift Broadband-Decoupled ^{1}H Spectroscopy 306
8.5.1 The Basis of Pure Shift Spectroscopy 307
8.5.2 Pseudo-2D Pure Shift 307
8.5.3 Real-Time Pure Shift 309
8.5.4 Pure Shift Refocussing Elements 309
References 313

9 Correlations Through Space: The Nuclear Overhauser Effect
9.1 Introduction 315
PART I THEORETICAL ASPECTS 317
9.2 Definition of the NOE 317
9.3 Steady-State NOEs 317
9.3.1 NOEs in a Two-Spin System 317
9.3.2 NOEs in a Multi-Spin System 324
9.3.3 Summary 329
9.3.4 Applications 330
9.4 Transient NOEs 335
9.4.1 Nuclear Overhauser Effect Kinetics 335
9.4.2 Measuring Internuclear Separations 336
9.5 Rotating Frame NOEs 337
PART II PRACTICAL ASPECTS 339
9.6 Measuring Transient NOEs: NOESY 339
9.6.1 The 2D NOESY Sequence 339
9.6.2 1D NOESY Sequences 346
9.6.3 Applications 349
9.7 Measuring Rotating Frame NOEs: ROESY 353
9.7.1 The 2D ROESY Sequence 353
9.7.2 1D ROESY Sequences 355
9.7.3 Applications 356
9.8 Measuring Steady-State NOEs: NOE Difference 359
9.8.1 Optimising Difference Experiments 361
9.9 Measuring Heteronuclear NOEs: HOESY 363
9.9.1 2D Heteronuclear NOEs 364
9.9.2 1D Heteronuclear Nuclear Overhauser Effects 365
9.9.3 Applications 366
9.10 Experimental Considerations for NOE Measurements 367
9.11 Measuring Chemical Exchange: EXSY 368
9.12 Residual Dipolar Couplings 371
9.12.1 Measuring RDCs 372
9.12.2 Applying RDCs 375
References 377

10 Diffusion NMR Spectroscopy
10.1 Introduction 381
10.1.1 Diffusion Coefficients and Molecular Size 382
10.2 Measuring Self-Diffusion by NMR 382
10.2.1 The Pulsed Field Gradient Spin-Echo 383
10.2.2 The Pulsed Field Gradient Stimulated-Echo 384
10.2.3 Enhancements to the Stimulated-Echo 385
10.2.4 Data Analysis: Regression Fitting 388
10.2.5 Data Analysis: Pseudo-2D Presentation 389
10.3 Practical Aspects of Diffusion NMR Spectroscopy 390
10.3.1 The Problem of Convection 390
10.3.2 Calibrating Gradient Amplitudes 397
10.3.3 Optimising Diffusion Parameters 397
10.3.4 Hydrodynamic Radii and Molecular Weights 401
10.4 Applications of Diffusion NMR Spectroscopy 403
10.4.1 Signal Suppression 403
10.4.2 Hydrogen Bonding 405
10.4.3 Host–Guest Complexes 405
10.4.4 Ion Pairing 408
10.4.5 Supramolecular Assemblies 409
10.4.6 Aggregation 411
10.4.7 Mixture Separation 412
10.4.8 Macromolecular Characterisation 413

10.5 Hybrid Diffusion Sequences 414
10.5.1 Sensitivity-Enhanced Heteronuclear Methods 414
10.5.2 Spectrum-Edited Methods 415
10.5.3 Diffusion-Encoded Two-Dimensional Methods (or 3D DOSY) 415
References 418

11 Protein–Ligand Screening by NMR

11.1 Introduction 421
11.2 Protein–Ligand Binding Equilibria 422
11.3 Resonance Lineshapes and Relaxation Editing 424
11.3.1 ^{1}H Relaxation-Edited NMR 426
11.3.2 ^{19}F NMR 428
11.3.3 Paramagnetic Relaxation Enhancement 429
11.4 Saturation Transfer Difference 430
11.4.1 The STD Sequence and Practicalities 432
11.4.2 Epitope Mapping by STD and DIRECTION 436
11.4.3 K_D Measurement by STD 437
11.5 Water-LOGSY 438
11.5.1 The Water-LOGSY Sequence 440
11.5.2 Water-LOGSY Practicalities 441
11.6 Exchange-Transferred Nuclear Overhauser Effects 441
11.7 Competition Ligand Screening 443
11.7.1 Competitive Displacement 444
11.7.2 Reporter Ligand Screening 445
11.7.3 ^{19}F *FAXS* 447
11.8 Protein Observe Methods 448
11.8.1 ^{1}H–^{15}N Mapping 448
11.8.2 ^{1}H–^{13}C Mapping 452
11.8.3 ^{19}F Mapping 452
References 454

12 Experimental Methods

12.1 Composite Pulses 457
12.1.1 A Myriad of Pulses 459
12.1.2 Inversion Versus Refocusing 460
12.2 Adiabatic and Broadband Pulses 461
12.2.1 Common Adiabatic Pulses 462
12.2.2 Broadband Inversion Pulses: BIPs 464
12.3 Broadband Decoupling and Spin Locking 465
12.3.1 Broadband Adiabatic Decoupling 467
12.3.2 Spin Locking 468
12.4 Selective Excitation and Soft Pulses 468
12.4.1 Shaped Soft Pulses 469
12.4.2 Excitation Sculpting 473
12.4.3 Chemical Shift Selective Filters 475
12.4.4 DANTE Sequences 477
12.4.5 Practical Considerations 478
12.5 Solvent Suppression 480
12.5.1 Presaturation 480
12.5.2 Zero Excitation 482
12.5.3 Pulsed Field Gradients 483
12.6 Suppression of Zero-Quantum Coherences 486
12.6.1 The Variable-Delay Z-Filter 486
12.6.2 Zero-Quantum Dephasing 487
12.7 Heterogeneous Samples and Magic Angle Spinning 489
12.8 Hyperpolarisation 491
12.8.1 *Para*-Hydrogen–Induced Polarisation 491
12.8.2 Dynamic Nuclear Polarisation 493
References 496

13 Structure Elucidation and Spectrum Assignment

13.1 ^{1}H NMR 500
13.2 ^{1}H–^{13}C Edited HSQC 501
13.3 ^{1}H–^{1}H COSY and Variants 503
13.3.1 Double-Quantum Filtered COSY 505
13.4 ^{1}H–^{1}H TOCSY and Variants 506
13.4.1 HSQC-TOCSY 508
13.5 ^{13}C NMR 508
13.6 HMBC and Variants 510
13.6.1 ^{1}H–^{13}C HMBC 510
13.6.2 ^{31}P and ^{1}H–^{31}P HMBC 512
13.6.3 ^{1}H–^{13}C HMBC Again 513
13.6.4 ^{19}F and ^{19}F–^{13}C HMBC 515
13.7 Nuclear Overhauser Effects 517
13.7.1 2D NOESY 517
13.7.2 1D NOESY 521
13.7.3 1D ^{19}F HOESY 522
13.8 Rationalization of ^{1}H–^{1}H Coupling Constants 523
13.9 Summary 525

Appendix 527
Subject Index 531